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SUPPLEMENTUM CXLI

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## NONSPECIFIC CAPSULAR SWELLING IN PNEUMOCOCCI

A SEROLOGIC AND CLINICAL STUDY

BY

*GUNNAR LÖFSTRÖM*

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 STOCKHOLM, SWEDEN.

# NONSPECIFIC CAPSULAR SWELLING IN PNEUMOCOCCI

## Errata.

Page	Line	Printed	Read
	2 from above	antibodies	red blood corpuscles
	14 " below	spirochetes.	spirochetes.
	21 " above	unneces-	unneces-
	20 " below	Na <sub>2</sub> PO <sub>4</sub>	NaH <sub>2</sub> PO <sub>4</sub>
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	13 " above	in cardiac	in acute cardiac
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TRANSLATED BY  
HELEN FREY

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STOCKHOLM

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*To My Wife*

would have been found in this case, if the analyses had been repeated at a later point of time.

### Discussion.

It can hardly be doubted that the necessary requirement for the production of these premortal electrolytic changes is a considerable degree of dehydration, leading at the same time to azotemia and pathological acids in the blood. The supply of liquids was minimal and the diuresis was impossible to measure. It is understandable that these changes will be most pronounced in case of apoplexy, where the patients are lying unconscious and receive no nourishment and where parenteral administration of liquids is usually omitted in view of the hopeless prognosis.

The cause must be supposed to be a selective loss of water, together with some retention of salt. Judging from the hemoconcentration the loss of water is percentually far greater than the increase in the total bases. The patients have been highly febrile and the insensible loss of water has certainly been considerable. Some have perspired very profusely and the sweat contained relatively little salt. One patient (No. 5) was given one litre of a 2.6 per cent sodium bicarbonate solution intravenously in two days. He got a cerebral thrombosis, became soporous and on the next day comatose. He died after four days. The conditions for an increase of the total bases, such as was seen in the first patients, should here be supposed to exist. In spite of intravenous injection of about 620 m.eq. of Na in the form of bicarbonate, which about corresponds to the whole content of Na in the blood, there came in this case a reduction of the serum Na. Neither was there any hemoconcentration, which probably explains why no premortal rise in the total bases occurred in this case.

The great degree of dehydration and the impaired vitality must be supposed probably through reduced filtration, to put the kidney partly out of action. The retention of urea and pathological acids may also be said to point in that direction. Perhaps there may be an increased reabsorption of Na through the tubules.

No increase of electrolytes was noted in patients with edema (Nos. 12, 13, 14). It is reasonable to suppose that the abundant supply of extracellular fluid serves as a depot which stands at disposal and prevents dehydration and reduction of the blood volume.

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## PREFACE.

Some years ago, while using the capsular swelling method of Neufeld in a study of acute pneumonia in the fourth medical service of St. Erik's Hospital, I observed a new serologic phenomenon which I called the nonspecific capsular swelling reaction. My studies of this phenomenon are described in the present communication.

The bacteriologic experiments were carried out at the State Bacteriologic Laboratory. I wish to express my warm thanks to its Director, Professor Carl Kling, for his kind interest in my work. Special thanks are also due to Doctors Sven Gard, Gunnar Norlin and Gunnar Olin at the same institute for valuable advice and help.

Besides the patients from the fourth medical service of St. Erik's Hospital numerous cases from other hospitals in Stockholm were studied. For this opportunity I wish to express my gratitude to the respective physicians in chief, Dr. Rolf Bergman, Professor Elis Berven, Dr. Hjalmar Eneström, Dr. Alf Gullbring, Professor Folke Henschen, Drs. Oskar Lindbom, Einar Perman, Ernst Sahlgren and Birger Strandell, and Professors Nanna Svartz and Josua Tillgren.

The electrophoretic experiments were done at the Institute of Physical Chemistry of the University of Uppsala. For this opportunity and for help with the analysis of the results, I am highly indebted to Professor Arne Tiselius. I also express my thanks to Dr. Harry Svensson of the same institute for doing the experiments.

The expenses of the investigation were defrayed in part by grants from the Swedish Society for Medical Research and the Pasteur Foundation of the Swedish Medical Society.

Stockholm, December, 1942.

*Gunnar Löfström.*



### Summary.

In several patients there was noted shortly before death a considerable increase of the total bases in serum, up to 184 m. eq./L. The content of chloride was practically normal. Together with this rise there was constantly found considerable hemoconcentration and an increase of N. P. N., as well as pathological acids in the serum.

The phenomenon was noted especially in patients dying of apoplexy, but also in one who died from a different cause. In fatal illnesses attended by considerable edema the total bases were normal or reduced and neither was there any hemoconcentration or increase of N. P. N. Probably because the large supply of extracellular fluid prevents dehydration.

It is supposed that the cause of these changes is dehydration with selective loss of water. In such patients, usually highly febrile, the insensible loss of water will be considerable and parenteral administration of liquid is in most cases omitted. One patient, in whom these electrolytic changes might have been expected, was given copious injections of liquid containing sodium bicarbonate. There was found no dehydration and likewise no increase of the total bases, in spite of copious administration of Na. On account of the dehydration the renal filtration probably becomes very much reduced so that the functioning of the kidneys as apparatus for regulation of the electrolytes becomes greatly restricted.

*Methods.* The total bases have been determined electrolytically by means of Herman Nielsen's apparatus. Chloride and alkali-reserve by Van Slyke's and the serum proteins by Howe's method.

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The point of departure for all more recent studies on pneumococci was the observation by Neufeld and Händel (73, 74) that their pneumococcic strains showed different serologic properties. They differentiated between three types of pneumococci in acute pneumonia. In 1913 Dochez and Gillespie (21) began a series of investigations at the Rockefeller Institute which in 1917 resulted in the monograph of Avery, Chickering, Cole and Dochez (5), a classic in this field of research. They divided pneumococci into the fixed types 1, 2 and 3 and placed the rest in a collective group 4, or as it was later called, X. Georgia Cooper and her co-workers (18, 19) divided this collective group into types 4—32. In subsequent pneumococcic studies strains were encountered which did not belong to the known types, and in 1938 Finland and Brown (30) showed that the Cooper types were not separate entities, but that there were some strains which showed overlapping serologic reactions. In 1939 Vammen (99) discovered two new types of pneumococci, 33 and 34, as well as three variants in type 9 and two in type 10. Kauffmann, Mørch and Schmith (53) then made a re-study of the whole type problem, and partly by analysis of partial antigen, they finally increased the number of pneumococcic types to fifty. Annabel Walter and co-workers (103) confirmed the results of the Danish authors and demonstrated still more types, increasing the total number to fifty-five.

The different types of pneumococci are identified with a method described by Neufeld (72) in 1902 but not used generally until the 1930's when serum treatment became popular. Neufeld describes the phenomenon observed by him as follows:

"Mischt man nun gleiche Theile von agglutinierendem Serum und Pneumokokken-bouilloncultur, sei es im Reagensglase, sei es durch inniges Verreiben, je einer Platinaöse Serum und Cultur auf einem Deckglase, so beobachtet man im hängenden Tropfen deutliche Quellungserscheinungen, die bei einem kräftig wirksamen Serum sofort oder nach einigen Minuten auftreten. Die einzelnen Kokken schwellen bis zum Doppelten und Dreifachen ihrer Grössen an, sie platten sich dabei an ihren Berührungsstellen, die sonst zugespitzt sind, gegen einander ab und die ganzen Contouren werden undeutlicher und verschwimmend. Bisweilen lässt sich deutlich erkennen, dass an diesem Quellungsprocess hauptsächlich die äusseren Schichten der Bakterienzelle betheiligt sind; sie wandeln sich in eine homogene glasige Masse um, während in der Mitte des Coccus ein dunklerer, runder Kern zu sehen ist."

The Neufeld capsular swelling has been more closely analyzed, mainly by Etinger-Tulczynska (25) in 1933 and is considered to be a reaction between the specific polysaccharide of the pneumococcic capsule and the serum antibodies. Whether the precipitate is deposited outside or inside the capsule is not known. The

size of the swollen capsule varies greatly in different types of pneumococci, but what is characteristic of the reaction is not so much the size of the capsule as the picture it presents, sharply outlined against the surrounding fluid (61).

The chemical basis for the variation in pneumococcic types is known through studies of Heidelberger and Avery (39, 40) and of Heidelberger, Goebel and Avery (42). They produced type-specific polysaccharides from the classical pneumococcic types 1, 2 and 3 and showed that they were composed of polysaccharide acids of high molecular weight and of characteristic composition for each type.

In 1930, Tillett, Goebel and Avery (93) produced a species specific polysaccharide from a rough type 2 strain. This polysaccharide, which differed serologically from known pneumococcic antigens, the type-specific capsular polysaccharide A and the species-specific nucleoprotein B, was studied clinically by Tillett and Francis (92) under the name "somatic C-substance". In 1931, Heidelberger and Kendall (46) isolated three polysaccharides from *Pneumococcus* type 4, of which one was specific for the type, the second probably identical with C-polysaccharide and the third a serologically inactive substance, closely related to chitin. The C-polysaccharide also differed chemically from the polysaccharides previously known by its content of phosphorus in addition to nitrogen and by being common to all the pneumococcic types. It occurred in both smooth and rough forms, bound to the pneumococcic body itself (45). Later on, specific, purified polysaccharides were produced from still further types of pneumococci and finally Brown (12) succeeded by ultrafiltration and alcoholic precipitation in producing chemically definable polysaccharides from all the thirty types of pneumococci in Cooper's classification.

Serologically active polysaccharides occur generally in the vegetable and animal kingdoms. They consist of both type specific and species specific antigens and have been used a great deal for classifying bacteria into types. For these questions I refer the reader to the handbooks of, for example, Topley and Wilson (97), limiting myself to the serologic observations of particular importance to my own investigations.

Forssman (33) showed in 1911 that serologically closely related substances occur in cells from biologically distinct animal species. The Forssman heterogenetic antigen is found in organs from

animals like the guinea pig, horse, cat and mouse, and stimulates the production of hemolysin against sheep antibodies on the immunization of rabbits. Heterogenetic antigens occur in the kidneys, liver, lungs, adrenal glands, testicles, skeletal muscles, lymph glands and serum. The Forssman antigen is lacking in several animals, like the rabbit, ox, sheep, rat, goose, pigeon and frog. Schiff and Adelsberger (85) showed that it occurs in human A blood corpuscles and that Forssman immune serum reacts with blood corpuscles A and AB, but not with O and B. Heterogenetic antigens were discovered in bacteria, first in dysentery, paratyphoid and leprosy bacteria and pneumococci and then in a large number of other bacterial species (86).

Splitting the Forssman antigen with alcohol produces a hapten which reacts with immune serum but which has no other antigenic properties. The protein component is nonspecific and can be replaced by pig serum. The hapten was originally thought to be a lipid, but the pure product proved to be soluble in water (58) and of polysaccharide nature (13).

Other heterogenetic antigens than the Forssman antigen have also been observed. Overlapping reactions between blood corpuscles from different animals occur, giving rise to different heterogenetic systems (52, 50, 106).

Other examples of heterogenetic reactions, according to Castaneda (15) are the Weil-Felix reaction in typhus fever, in which reaction *Proteus* X19 and *Rickettsia prowazeki* prove to have chemically similar antigens, and the Wassermann reaction in which Eagle and Hogan (23) showed, by absorption experiments with the Reiter strain closely related to the syphilis spirochetes, that the Wassermann reagin contains substances with serologic properties similar to the antigenic components of the syphilis spirochetes.

Finland and Curnen (31) found that antipneumococcic serum type 14 from horses agglutinates the blood corpuscles of all human blood groups. The specific type 14 polysaccharide shows close chemical resemblance to the blood group A substance isolated from peptone (37) and, according to Beeson and Goebel (7), absorption with the type 14 polysaccharide removes both the agglutinins for human blood corpuscles and the precipitins for the A substance from type 14 antipneumococcic horse serum. Absorption with the A substance does not cause an appreciable decrease in the type 14 antibodies, but does reduce the serum's

capacity to agglutinate human blood corpuscles. Absorption with human blood corpuscles diminishes the content of precipitin against the A substance. The authors pointed out that the serologic reaction between the specific substances from type 14 and blood group A appears to be due to a similarity in the chemical constitution of the specific polysaccharides.

Overlapping serologic reactions also occur between antigens from the following groups, among others: B. Friedländer and *Pneumococcus* type 2 (16), yeast and *Pneumococcus* type 2 and 3 (41), and specific polysaccharides from gonococci and meningococci on the one hand and *Pneumococcus* type 3 on the other (71).

In addition, overlapping reactions have been observed between some of the different types in the pneumococcus group. Table 1 records the results obtained by different authors (66, 100, 105) in the production of type specific rabbit serum. A high homologous titer was obtained against the immunizing bacteria, but overlapping reactions against other types of pneumococci were also observed.

Certain of the overlapping reactions shown in the table are of particular interest to the following investigations. Type 16 serum reacts with type 28, type 27 serum with type 28 and type 28 serum with types 16, 27 and 29. Walter and her co-workers (103) demonstrated similar overlapping reactions. Type 16 serum reacts with types 11A, 21 and 28, type 27 serum with type 32 and type 28 serum with types 15A, 16, 27 and 29.

The polysaccharide antigen is characterized on the one hand by a high degree of serologic specificity, on the other hand by an abundant occurrence of heterogenetic reactions. Landsteiner (55) explains this phenomenon as follows: "As explanation for the frequent occurrence of heterogenetic reactions among cell antigens the suggestion may be offered that these reactions depend upon the special chemical nature of the haptens. Whereas proteins are built up from many different amino acids, the carbohydrates which determine the specificity of numerous bacterial antigens have fairly high molecular weight, yet, as far as is known, each of them contains but few different components. Therefore, the presence of the same or similar constituents in different carbohydrates and the probability that these components or simple combinations thereof are significant for the specificity, offers a reasonable explanation for the heterogenetic reactions in bac-



teria . . . Heterogenetic reactions do not imply that one and the same substance is present in the similarly reacting materials, although this is often held. It has been demonstrated in absorption and immunization experiments, and also by comparing various immune sera, that Forssman reactions, which possibly depend on the presence of a small characteristic grouping, are due to similarity rather than to identity of the substances."

Table 1. — The occurrence of overlapping reactions between pneumococci of types 1 to 32 (Cooper) in the immunization of rabbits.

Homologous types	O v e r l a p p i n g   t y p e s		
	A u t h o r s		
	Lyall and Odell	Welch, Borman and Mickle	Vammen
1	—	—	—
2	20	17, 20, 27	20
3	—	—	8
4	—	9	—
5	—	2, 21, 27	—
6	—	—	11
7	—	16, 18, 22, 28, 31	—
8	19	3, 13, 19	3, 19
9	—	—	—
10	20, 29	20, 29	—
11	—	—	—
12	—	13	—
13	17, 29	—	—
14	—	8	—
15	—	11	11, 14
16	—	28	28
17	13	13, 29	15
18	28	1, 16	1, 16, 23
19	8	8	2, 8
20	2, 10, 31	10, 21, 31	2, 13, 31, 33
21	—	—	7, 27
22	—	—	2, 20, 31
23	32	16, 32	7, 18, 32
24	—	—	11
25	—	10, 20	—
27	—	—	28, 32
28	18	16, 27, 29	16, 18, 27, 29
29	10, 13	10	13, 28
31	20	20	20, 22
32	23	23, 27	23, 27

## CHAPTER 2.

**The Nature of Antibodies.**

The mechanical explanation for antibody reactions, expressed in Ehrlich's side-chain theory (97), is now only used for schematic representation, but despite increased biochemical knowledge the fundamental problems regarding the nature and origin of antibodies still await a definitive solution. Although a great many antigens are chemically defined, the exact chemical nature of the antibody molecule is still obscure.

It is known that the active components of the antibodies are of globulin nature, or at any rate intimately related to globulin, and as yet no one has succeeded in producing protein-free antibodies (22). According to Breinl and Haurowitz (11), among others, the antibody globulins are formed in the reticulo-endothelial system, together with the normal globulins. Injected antigen is also taken up in this system and these authors assume "dass das abgelagerte Antigen den Aufbau der Serumglobuline aus ihren Aminosäure-Bausteinen stört, so dass an Stelle der normalen Serumglobuline neuartige Globuline entstehen, welche der determinanten Gruppe des Antigens räumlich und hinsichtlich Verteilung ihrer Ladungen angepasst sind".

Before going on with this discussion, certain elementary results of modern antibody research must be recorded. The former classification of antibodies into precipitins, agglutinins, lysins, tropins, amboceptors and so on, is now generally regarded as unjustifiable (29, 43, 44, 29, 81). The different names are now considered to express different modes of behavior of the same serologic substance. Landsteiner (55) summarizes his opinion as follows: "In brief it can be said that frequently one antibody can cause reactions different in appearance, as the agglutination of bacteria by the same antibodies that give precipitin or complement fixation reactions with bacterial polysaccharides. On the other hand, a cell containing several antigenic constituents will give rise to at least as many — if not more — antibodies as there are antigenic components, and these may well differ in their effects on the whole antigenic complex."

Antibodies occur as immune antibodies and as normal antibodies. The serologic properties of the former are well known and will only be briefly recapitulated. An immune antibody develops

after antigenic stimulation and reacts specifically with its antigen and possibly heterologously with certain antigens of closely related chemical nature. After antigenic stimulation a certain amount of time elapses before the appearance of antibodies in the blood. The concentration of antibodies increases successively and reaches its maximum after a certain length of time, when it begins to diminish, first rapidly and then more slowly (98). If the antigenic dose is repeated after a time, the antibodies develop more rapidly than after the primary dose. This "secondary stimulus" (Glenny and Südmersen, 36) also leads to a higher concentration.

Normal antibodies have the same serologic properties as immune antibodies to a certain extent, but their occurrence in the serum is not preceded by any known antigenic stimulation. They generally have a low titer and are difficult to remove by absorption (see Topley and Wilson). The specificity of normal antibodies is debated. A number of investigations with absorption (24, 70) indicate that they are specific and likewise a number of more recent experimental observations (65, 67). Gordon and Carter (38), however, are inclined to believe that the effect of normal antibodies is due to nonspecific factors in the serum.

There has been a great deal of discussion about the origin and mode of development of normal antibodies. The newly born infant possesses a passive immunity from its mother, obtained through the placenta, and during the suckling period antibodies are transferred to it with the milk, but infant serum is otherwise poor in antibodies. During the first years of childhood their number increases, together with the globulin concentration in the serum (97). The common explanation for this is that unapparent infections cause the formation of homologous and to some extent heterologous antibodies. This explanation is decidedly unsatisfactory as regards a number of antibodies, however, and it is probable that antibodies are not all formed in the same way. Another theory is that antigenic material absorbed through the digestive tract or other mucous membranes gives rise to antibodies. Antibodies are probably also caused primarily by the antibody-producing apparatus, without any external stimulation (see Topley and Wilson). Landsteiner (55) believes "that one may distinguish two kinds of antibodies in apparently normal animals, acquired and physiological, and the conclusion may be drawn that normal serum contains a complete assemblage

of substances, which though not adjusted to any one antigen, in other respects resemble the antibodies produced by immunization".

I shall now mention a few studies regarding normal antibodies against the bacteria of the pneumococcic group. Robertson and his co-workers (83) found that human sera contained antibodies against one or more of types 1, 2 and 3. They also observed that during the first day of acute pneumonia the serum sometimes also contained mouse-protective antibodies against the infecting bacteria. The normal serum of certain species of animals has a strong bactericide action on pneumococci of different types (67, 102).

Faber (26) demonstrated agglutinins against one or several pneumococcic types in sera from 18 per cent of a series of healthy persons. Sutliff and Davies (87) pointed out that the normal antipneumococcic antibodies which develop during the first years of childhood have no connection with the different types of pneumococci occurring in the throat of the same children.

This leads to the question of "auto-antigen". Extracts of tissues, except for nervous tissue and the eye lens, transudates and exudates lack antigenic properties for the same organism (107). Some authors believe, nevertheless, that as soon as a tissue is pathologically altered, it may become "foreign" to the body and act as an auto-antigen (47, 49, 60). The following statement by Lehmann-Facijs and Loeschke (60) is of interest when considering the results of the present investigation: "Wie beim Karzinom, so bestand auch bei der Tuberkulose die Hauptschwierigkeit darin, dass die nachzuweisenden Körper zur Gruppe der Auto- oder Isoprecipitinen gehören, die bekanntlich infolge ihrer geringen Titerhöhe im Gegensatz zu den Kraus und Uhlenhuthschen Precipitinen serologisch schwer zu erfassen sind."

The present investigation is connected to a certain extent with the problem of the effect of nonspecific stimuli upon immunity and resistance to infection. The observations made in this field of study are based on experiments which are often difficult to judge from an experimental standpoint and difficult to explain from a theoretical standpoint. I should like to go over some fundamental concepts in this field. Various stimulants, such as colloidal sulphur, protein solutions and bacterial suspensions nonspecifically activate antibodies present in a low titer in the body. This anamnestic reaction (Conradi and Bieling, 17) is considered to

play a part in nonspecific stimulation therapy. Characteristic of it is that the antibodies appear after a very short latent period and their effect passes over quickly. It has been considered possible to produce generally increased resistance to infections by nonspecific stimulation. A large amount of literature has grown up around this problem (80). Orskov and Kauffmann (78) coined the term "promunity" for one type of heightened resistance and drew attention to its short life in comparison with ordinary immunity, which develops later and decreases gradually.

There have been many attempts to explain the nonspecifically induced resistance to infection, but the definitive solution is still wanting (80). The following are a few of the theories. Weichardt (104) looks upon the nonspecific protective effect as a protoplasmic activation of quantitative but not qualitative nature. Sachs (84) assumes that the injection of colloids and nucleic acid acts on the proteins of the organism, and that their decomposition produces omniscellular reactions. As long ago as 1894 Vaughan (101) presented the theory that injections of nucleic acid causes an increased resistance due to activation of the tissue.

### CHAPTER 3.

#### **Earlier Research on Nonspecific Serologic Reactions During Acute Stages of Disease in Man.**

During acute disease, the blood serum undergoes considerable changes. The changes vary according to the etiology of the disease, but some are common to different diseases or conditions of disease.

The decrease in the suspension stability of the red blood corpuscles as measured with the sedimentation reaction (27, 28) is one of these phenomena. Fåhræus considered that the reduced stability, which causes an elevated sedimentation rate, was due to an increase of the globulins in the plasma, especially fibrinogen.

The globulins often increase during infectious diseases, diabetes and some kidney diseases, during experimental immunization and after the injection of nonspecific stimuli (28).

Tiselius (94, 95, 96) divided serum into four fractions by means of electrophoresis. The most quickly moving component corre-

sponded on the whole to the albumin of the serum. The three other fractions, which moved more slowly and which corresponded approximately to the globulin, were called  $\alpha$ ,  $\beta$  and  $\gamma$  globulins.

Blix (8) made an electrophoretic analysis of sera from cases of acute disease, and showed that the concentration of the  $\alpha$  globulin practically doubled during acute pneumonia, while the other fractions remained largely unchanged. Normally, he found, the  $\gamma$  fraction accounted for about three-quarters of the globulin and the  $\beta$  and  $\alpha$  globulins for one-sixth and one-14th to one-15th, respectively. Longsworth, Shedlovsky and MacInnes (64) found that the albumin/globulin quotient was generally lower in pathologic sera than in normal sera and that the  $\alpha$  globulin/albumin quotient was higher than normal in sera from febrile patients. The  $\gamma$  globulin/albumin quotient was elevated in aplastic anemia and rheumatic fever, and likewise the  $\beta$  globulin/albumin quotient in a number of diseases, especially nephrosis.

In 1939 Nissen (76) drew attention to the change in the content of plasma fibrinogen during acute pneumonia. It is highly elevated, especially in severe cases, and drops to normal as the patient recovers.

In addition to free proteins, normal serum also contains polymeric carbohydrate complexes both in the serum albumin and in the globulin (82). Nilsson (75) showed that the content of this carbohydrate becomes elevated during acute pneumonia. According to Friedeman and Sutliff (35), the same elevation occurs during acute bacterial infections, acute nephritis and hypertension during pregnancy, and also in some cases of tuberculosis. The newly formed polysaccharides differ from those occurring normally in that they can be broken down into simple sugars and fermented to lactic acid by rapidly growing strains of pneumococci.

Little research has been done on the behavior of normal antibodies during acute disease. Wulff (108) observed an increase in the bactericide action of the serum on certain bacteria. His observation was gone into thoroughly by Hjorth (48). The lysin, which Hjorth called X-lysin, appears in many diseases especially ones with high fever, and is directed particularly against gram-negative cocci but also against a number of staphylococcic and pneumococcic strains. Patients with a normal temperature may also have X-lysin in their blood, especially ones suffering from

malignant tumor. The X-lysin only appears after powerful irritation of the organism, either in the form of infection or some other fever-causing agent or "auf einen Reiz aus dem Organismus selbst". It is not absorbable and is contained in a borderline fraction between globulin and albumin, with the greater part in the albumin. It consists of two components with different sensitivity to heat. Hjorth said that it shows great likeness to  $\beta$ -lysin (79) and to bactericide leukocytic substances.

Tillett (90) found in 1937 that serum from acutely ill patients had an increased bacteriolytic action upon hemolytic streptococci *in vitro*.

The occurrence of antifibrinolysin has been studied, particularly in connection with hemolytic streptococcic infection for which it is considered specific (91). Boisvert (10) showed, however, that this antibody also occurs nonspecifically in non-streptococcic infections, for example, during acute phases of pneumonia. Unlike the specific reaction, where the content of antifibrinolysin increases as the patient recovers, the nonspecific reaction is most powerful at the peak of the acute symptoms, and decreases and disappears together with the latter.

Tillett and Francis (92) reported that precipitins against the "somatic C-substance" of pneumococci (p. 11) occurred in high titer in sera from patients undergoing acute phases of infectious disease. Both streptococcic and pneumococcic infections gave rise to this reaction, and Tillett and Francis believed that it was a question of an anamnestic reaction against a species-specific polysaccharide, common to gram-positive cocci.

Continuing the research of Tillett and Francis, Rachel Ash (4) found that precipitins against the C-fraction occurred irrespective of whether the disease was caused by gram-positive or gram-negative bacteria. She could not produce the reaction in syphilis and only irregularly in tuberculosis. She wrote: "In many instances there is a relationship between fever, increased rate of sedimentation and this phenomenon of precipitation but the relationship is not constant. The phenomenon is not one of maturation related to age or to previous infection since it may be observed in early infancy".

In 1934 Francis and Abernethy (34) showed that the occurrence of anti-C-substance in the serum was accompanied by skin hypersensitivity, if the C-polysaccharide was injected intracutaneously. This observation was confirmed later by Finland and Dowling

(32), and the agreement between the serologic and cutaneous reactions was still further stressed in a publication by Abernethy and Francis (3).

Abernethy (1) found that the reaction could be produced in the monkey but not in the rabbit or mouse.

In 1941 Abernethy and Avery (2) as well as MacLeod and Avery (68) published investigations which threw still further light on the reaction between the C-polysaccharide of pneumococci and the serum during the acute stages of bacterial infection, which the authors called "acute phase" serum. The following conclusions were drawn: The reactive component of acute phase serum is of protein nature and bound to the albumin fraction. Heating to over 65 C. deprives it of its reactive power, and the presence of calcium ions are necessary for the precipitation. The C-reactive protein has good antigenic properties, differing in this respect from earlier known immune proteins. The immunization of rabbits with "acute phase" serum gives an antiserum by means of which one can detect minimal amounts of "acute phase" serum in humans or monkeys.

I showed in 1939 (62) that under certain conditions, a substance occurred in blood serum which caused swelling of the capsules of pneumonococci of types 27 and 28, sometimes also of types 9, 10, 16, 17, 18, 21 and 23. The best results were obtained by using about twenty times greater dilution of the vaccines than in the regular slide agglutination method. Agglutination followed when the swelling was marked.

Nonspecific capsular swelling seemed to occur regularly in pneumonia, less regularly in acute upper respiratory infections. In 120 healthy recruits just joining their regiment and coming from different parts of the country the reaction was absent. During an epidemic of acute respiratory infection the swelling was observed in the sera of about one third of 157 soldiers. This percentage was independent of the elevation of the body temperature. Complications developed chiefly among the soldiers whose sera showed this type of swelling. In the group with fever above 39 C., twelve complications (ten cases of pneumonia) occurred among the 36 per cent which showed the reaction as opposed to one complication among the 64 per cent which did not. Eighteen cases of lobar pneumonia and bronchopneumonia all showed the reaction. It was demonstrable in the first blood sample taken after admission to the hospital, viz. on the first



to seventh day. In 12 cases in which the disease ran a smooth course the swelling disappeared between the sixth and twelfth day. In the other cases it persisted and complications developed or the disease ended fatally. In 1942 I published a further report (63) in which I demonstrated that nonspecific capsular swelling substance was present in the serum during acute stages of disease, infections of varying origin and non-bacterial diseases characterized by absorption of disintegrating tissue. In the same year Carlens (14) showed with my method that nonspecific capsular swelling occurred to a large extent during the acute phase of acute otitis, and that the reaction remained positive a longer time in the cases complicated with mastoiditis than in the uncomplicated cases. Carlens also showed that the reaction occurred within twenty-four hours after sterile operations on the knee, in which every form of active infection could be ruled out. He also demonstrated that even nonspecific stimuli, such as sulphur suspended in oil (Allergol), caused the reaction and pointed out that this fact could possibly be used to check up on the nonspecific stimulating effect of these agents in, for example, allergic diseases (personal communication).

## CHAPTER 4.

### The Serologic Properties of Nonspecific Capsular Swelling.

#### Methods.

The pneumococcic strains used in my experiments were with but few exceptions cultivated at the State Bacteriologic Laboratory and their type according to Cooper determined with sera from Lederle's laboratories in U. S. A. It was not considered necessary to use the revised classification of Kauffmann and co-workers. It makes little difference to my results if the partial antigens of certain of the types used do not agree entirely with the international type sera which may be worked out later.

The pneumococcic suspensions were either prepared directly after the isolation of the bacteria, or after the strains had been kept dried in a refrigerator. Virulent strains with good capsular forming capacity were used. The bacteria were allowed to grow for six to eight hours on hormone broth to which 0.1 per cent glucose had been added, and were killed by treatment with a 0.7 per cent solution of formaldehyde (2 per cent commercial formol) for eighteen hours at room temperature. After centrifuging, the bacteria were suspended in a physiologic salt solution containing 0.15 per cent formaldehyde. Bacterial suspensions prepared in this way can be kept for several years without losing their reactive properties.

The nonspecific capsular swelling reaction was carried out with *Pneumococcus* type 27 (strain S. B. L. 665/37 and 27 L, the latter obtained from Dr. Kauffmann at the Danish State Serum Institute). In a number of cases the investigations were complemented with type 16 (strain S. B. L. 536/37 and strain S. B. L. 683/41) and type 28 (strain S. B. L. A313/39) and comparative studies were made with the other pneumococcal types 1 to 32.

As a rule, the slide method was used for demonstrating capsular swelling and agglutination. It was carried out according to the Neufeld method, as described by Löfström (61).

A normal loopful of bacterial suspension is placed on a slide and mixed with a loopful of serum, after which a loopful of Löffler's alkaline methylene blue diluted 1:2 is added for staining. A cover glass is put on and half an hour later the preparation is ready for observation under the microscope. An immersion lens and magnification of at least one thousand times are used. Two phenomena are looked for in the microscope, firstly, capsular swelling, which generally occurs instantaneously, and secondly, agglutination, which often requires a certain length of time to become stabilized.

As regards the density of bacteria in the suspension, it is desirable to have as few bacteria as possible per unit of serum in order to obtain as sensitive a reaction as possible, and yet enough bacteria per field of vision so as not to prolong the microscopic examination unnecessarily. A suitable density was found to correspond to a twentyfold dilution of Burrough's and Wellcome's opacity tube number 3, which gave one to five bacteria per field of vision.

In a previous communication I used certain signs for capsular swelling and agglutination, but I have since had to revise and adapt them to suit the lesser bacterial density used. The following is a list of the revised signs.

+, 4—6 clumps containing 2—3 diplococci or chains.

++, several clumps of 4—6 agglutinated diplococci or chains.

+++, flocculation of most of the bacteria in large or small clumps.

c, distinct capsular swelling.

(c), distinct capsular swelling without clear demarcation from surrounding fluid.

The following points should be kept in mind when performing the reaction and judging the results.

The suspensions should be shaken carefully each time before use. It is easier if a couple of glass beads are put in each flask.

The suspensions should be kept at refrigerator temperature, the staining fluid at room temperature. The latter should be clear and the platinum loop must not be warm when inserted.

Attention should be given to free bacteria and clumps; clumps which have sunk or become suspended on impurities should be disregarded.

Agglutination is always uniformly distributed. Large clumps in an otherwise non-agglutinated preparation should be disregarded and likewise agglutination close to the edge of the cover glass.

## The Content of Nonspecific Capsular Swelling Substance in the Serum.

The content of capsular swelling substance is low in comparison with that of immune antibodies. Sera with a high content seldom bind more than 1,000 million bacteria per milliliter of serum. Titration

of capsular swelling is generally done in small test tubes and the reading done in the microscope (26). Another way is to titrate the serum on a slide with a constant amount of serum against increasing bacterial concentrations.

The sensitivity of these two methods was compared. In the slide method the previously described basic bacterial suspension was called density 1 and the corresponding content of nonspecific capsular swelling substance titer 1. Densities 2 to 64 were then obtained by successive doubling of the bacterial concentration in density 1. Density 16 corresponds to about 1,000 million bacteria per milliliter.

For titration in test tubes, constant bacterial concentrations, densities 1 and 8, were used against decreasing serum dilutions; the first tube with undiluted serum gives the titer 1 : 2. When using the lower concentration of bacteria the titer 1 : 2 corresponds to the slide agglutination titer 1. The tubes were placed in a water bath of 50 C. for one hour and then kept in the refrigerator over night. The reaction was determined microscopically for the series in which bacterial density 1 was used and macroscopically in the case of bacterial density 8. For the microscopic determination one drop from each tube was put on a slide. A drop of methylene blue was added, a cover glass laid on and the microscopic reading done. The preparation has the same appearance as in direct slide agglutination. Capsular swelling and sometimes agglutination was observed in the drops from some of the tubes.

Table 2 gives a comparison between slide and test tube titration in ten different sera.

**Table 2. — Slide titration with *Pneumococcus* type 27 of nonspecific capsular swelling substance using a constant amount of serum against increasing amounts of bacteria compared with test tube agglutination using decreasing amounts of serum against constant amounts of bacteria.**

Sera	Slide titration Titer	Capsular swelling in test tubes Bacterial density 1 Titer	Agglutination in test tubes Bacterial density 8 Titer <sup>1</sup>
1	2	$\frac{1}{4}$	2, 0
2	64	$\frac{1}{8}$	—
3	16	$\frac{1}{8}$	—
4	8	$\frac{1}{8}$	2, 0
5	16	$\frac{1}{16}$	2, 0
6	2	$\frac{1}{1}$	0
7	4	$\frac{1}{8}$	2, 0
8	16	$\frac{1}{8}$	2, 0
9	16	$\frac{1}{4}$	2, 0
10	16	$\frac{1}{1}$	3, 2, 1
11	16	$\frac{1}{16}$	2, 0

<sup>1</sup> The first dilution used in test tube agglutination was  $\frac{1}{2}$ . The agglutination observed macroscopically was graded as follows: 3 = large clumps, not fusing together into a plaque; 2 and 1 = still finer clumps and less typical agglutination; 2, 0 = agglutination of grade 2 with the dilution  $\frac{1}{2}$  and none in the  $\frac{1}{1}$  dilution.

The table shows that the test tube method gives rather unsatisfactory results in the case of both the larger and the smaller bacterial amount. Macroscopically typical agglutination was not observed in any of the test tube series. On microscopic analysis the method gave a slightly lower titer than the slide agglutination. Without going into the cause of the lack of conformity between the capsular swelling and agglutination, I should like to point out that, as titration with a constant amount of serum against increasing amounts of bacteria did not prove inferior to the test-tube method for titrating the content of nonspecific capsular swelling substance, I used the first method throughout my work.

In one section of my work I titrated the specific antibodies in test tubes against bacterial density 16.

### Sensitivity to Temperature of Nonspecific Capsular Swelling Substance.

The effect of temperature on the non-specific capsular swelling substance was tested partly by long storage in a cold room ( $-2$  to  $-4$  C.) and at room temperature, and partly by heating to  $56$  to  $70$  C. for different lengths of time.

Table 3. — The effect on nonspecific capsular swelling substance of storage at  $-2$  to  $-4$  C. for one week, tested with *Pneumococcus* type 27.

Sera	Original N.C.S. titer	Daily tests during week of cold storage						
		1	2	3	4	5	6	7
12 . . . . .	1	0						
13 . . . . .	1	0						
14 . . . . .	1	0						
15 . . . . .	1	0						
16 . . . . .	1	1		0				
17 . . . . .	2					0		
18 . . . . .	2	2		1		0		
19 . . . . .	2				2			2
20 . . . . .	4		4		1			1
21 . . . . .	4			4		4		1
22 . . . . .	4			4		1		1
23 . . . . .	4				1			1
24 . . . . .	4		4		1			1
25 . . . . .	4		2		1			1
26 . . . . .	8			8		8		1
27 . . . . .	8				1			1
4 sera . . . .	8							8
6 sera . . . .	16							16

N.C.S. = Nonspecific capsular swelling substance.

Table 3 shows the sensitivity to cold temperature and table 4 to ordinary room temperature of sera with varying contents of non-specific swelling substance. All the sera were stored in a cold room from the time they were taken until the day for the first capsular swelling test.

Table 4. — The effect on nonspecific capsular swelling substance of storage at room temperature (18–22 C.), tested with *Pneumococcus* type 27.

Sera	Original N.C.S. titer	Daily tests during week of storage at room. temperature						
		1	2	3	4	5	6	7
28 . . . . .	1	0						
29 . . . . .	1	0						
30 . . . . .	2	2	0					
31 . . . . .	2	2		1		1		0
32 . . . . .	2		2		1			1
33 . . . . .	4	4	4	2		0		
34 . . . . .	4		4	0				
35 . . . . .	4		0					
36 . . . . .	4			2		2		1
37 . . . . .	4		4		4		2	
38 . . . . .	4		2		2			2
39 . . . . .	4		2		1			0
40 . . . . .	8	8	8	4		4 <sup>1</sup>		4 <sup>1</sup>
41 . . . . .	8	4		1	0			
42 . . . . .	8		8	8		4		4
43 . . . . .	8			4		4		4
44 . . . . .	8		4		4		2	

<sup>1</sup> Agglutination but not capsular swelling.

It is seen that sera with a low content of the substance are more quickly weakened than sera with a high titer. Considering the uncertainty attending titration of this kind, the conclusion may be drawn that sera with titers equal to or exceeding 4 can stand a week's storage in the refrigerator without any appreciable weakening. In one case with a titer of 4, however, the titer dropped to 1 in a week. Sera with titers of 1 and 2 are generally weakened so much that they grow negative within a week.

Room temperature weakens still more the nonspecific capsular swelling substance. Weak sera are destroyed most quickly at this temperature but even sera with a high content of the substance often showed distinct weakening during the week of observation; in some cases the agglutinating capacity outlived the capsular swelling ability.

Sera kept frozen retain their nonspecific capsular swelling capacity practically unchanged for several years.

The effect of heat was determined in two ways, firstly, by heating at temperatures of 56, 60, 65 and 70 C. for half an hour and, secondly, by heating at 56 C. for a longer time. Table 5 shows the results of the first experiment and table 6 of the second.

Table 5. — The effect on nonspecific capsular swelling substance of heating at different temperatures for half an hour.

Sera	Tested with type 27						Tested with type 28				
	N.C.S. titer	Temperature (C.)					N.C.S. titer	Temperature (C.)			
		56°	60°	65°	70°	75°		56°	60°	65°	70°
45 . . . . .	1	0	—	—	—	—					
46 . . . . .	2	1	1	0	—	—					
47 . . . . .	8	8	4	4 <sup>1</sup>	2 <sup>1</sup>	—					
48 . . . . .	8	—	4	2	0	—					
49 . . . . .	1	1	0	—	—	—	1	1	0	—	
50 . . . . .	16	16	8 <sup>1</sup>	8 <sup>1</sup>	0	—	16	16	16 <sup>1</sup>	4 <sup>1</sup> 0 <sup>1</sup>	
51 . . . . .	8	8	8	4 <sup>1</sup>	0	—	16	16	4	4 <sup>1</sup> 0 <sup>1</sup>	

<sup>1</sup> Serum coagulated.

Table 6. — The effect on nonspecific capsular swelling substance of heating at 56 C., tested with *Pneumococcus* type 27.

Sera	N.C.S. titer	½ hr.	1 hr.	2 hrs.	4 hrs.	8 hrs.	24 hrs.
45 . . . . .	1	0	—	—	—	—	—
49 . . . . .	1	1	(1)	(1)	0	—	—
50 . . . . .	16	16	16	16	8	4	0
51 . . . . .	8	8	4	4	2	0	—
52 . . . . .	4	2	2	1	—	0	—

Table 7. — The effect on nonspecific capsular swelling substance of heating for one half hour at 65 C., tested with *Pneumococcus* type 27.

Original N.C.S. titer	No. of cases	Titer after heating ½ hr. at 65 C.					No. of sera in which heat caused at least 50 % loss of the substance	
		neg.	1	2	4	8		
1 . . . . .	14	11	3				11	
2 . . . . .	20	15	2	3			17	85.0
4 . . . . .	33	8	4	14	7		26	78.8
8 . . . . .	18			3	6	9	9	50.0
16 . . . . .	3					1	1	

These two tables confirm the observation that when there is a high content of the substance in the serum, it is more resistant to heat than when there is a low content. An interesting point is that the substance remains active even after the serum has coagulated.

The resistance of nonspecific capsular swelling substance to half an hour's heating at 65 C. was studied in a number of blood specimens, and the results are seen from table 7.

The table shows that the titer dropped to half its original value in 50 per cent of the cases where the original titer was 8, against 78.8 per cent with titer 4 and 85 per cent with titer 2.

### **The Occurrence in Non-Immune Sera of Substances Which Cause Agglutination and Capsular Swelling of Other Pneumococcic Types Besides Type 27.**

A number of sera were tested for their capacity to agglutinate and cause capsular swelling of other types of pneumococci in addition to type 27. Fifteen sera were chosen from cases of different diseases in which the nonspecific capsular swelling was positive and fifteen sera from ill and healthy persons in which the reaction was negative with type 27. The ordinary slide method was used with bacterial density 1 for all the types. The upper half of table 8 shows sera causing nonspecific capsular swelling, the lower half the sera which did not.

It is seen that the sera in the table had the capacity to react with several types of pneumococci. The content of the reacting substance was low, so low that earlier investigations of mine, in which a mixed vaccine with a bacterial density of about 16 was used for each type of pneumococcus, rarely revealed any reactive substance apart from immune antibodies. Whether the aggregation of pneumococci, graded with +, can be considered an antibody effect in the real sense is difficult to say. It is certain, however, that it constitutes an effect of the serum, since bacterial suspensions themselves never show any aggregation if they are well shaken before use.

Types 16, 27 and 28 behaved differently from the rest of the pneumococcic types examined (table 8). Type 16 reacted with all the sera which contained nonspecific capsular swelling substance, in many cases with distinct and typical capsular swelling, in others with a reaction of type (c). Type 16 also reacted fairly often with sera from healthy persons and with other sera not causing nonspecific capsular swelling, but in these cases with only agglutination. Type 27 showed typical nonspecific capsular

swelling and reacted with only one of the sera not containing nonspecific capsular swelling substance. Type 28 behaved in the same way as type 27 on the whole, but showed less distinct capsular swelling. The other types which reacted appeared to do so irrespectively of the presence of nonspecific capsular swelling substance, with the possible exception of some types, especially type 10, which reacted slightly more often with sera containing the substance. Thus it is possible with this experimental arrangement to differentiate between different reactive substances occurring in serum, the nonspecific capsular swelling substance which always reacts with types 16, 27 and 28, apart from occasional exceptions in the case of type 28, and substances of the nature of normal antibodies which react with a different number of types in different sera.

To complement the foregoing investigation, serial experiments were done in three cases (83, 84 and 85) to see how the nonspecific capsular swelling substance and normal antibodies varied during fever produced by injections of sulphur. These experiments are taken up in table 9.

*Case 83* was one of a 54-year-old woman treated at the fourth medical service of St. Erik's Hospital for organic heart disease and cerebral thrombosis. Sulphur injections were given after all the clinical signs had subsided and the heart was well compensated. The sulphur preparation used was Sulfosin (Leo) which contains 10 mg. of sulphur per milliliter of oil. The plan of dosage is seen from the table. Nonspecific capsular swelling substance appeared the first or second day after the injections. Types 16 and 27 reacted parallely on the whole. Type 28 gave a positive reaction before the injections were begun, probably due to the presence of normal antibodies. Types 2 and 14 reacted positively in all the tests, because of the occurrence of normal antibodies.

*Case 84* was one of a 74-year-old shoe salesman who had suffered from stomach ulcer and asthma for about ten years. In April of 1940 gastro-enterostomy was performed. On Sept. 10, 1941 he fell ill suddenly with a large gastric hemorrhage and was admitted the next day to the fourth medical service of St. Erik's Hospital. Blood analysis then showed 4,300,000 red blood cells, 9,500 white blood cells and 70 per cent hemoglobin. During the first part of his hospital stay, he suffered from severe asthma, which decreased gradually after a time but increased again at the end of October. Blood studies at that time showed slight anemia but the temperature was normal. Injections of sulphur were given for desensitizing purposes on the 20th, 23rd and 26th of October, in doses of 0.2, 0.4 and 0.8 Ml. The preparations used was Allergol (Astra) which contains 5 mg. of sulphur per milliliter of



Table 8. — Testing of sera showing a positive nonspecific capsular  
against pneumococci of

(The letters after the case numbers refer to the diagnosis: A = healthy persons, meningitis, G = septicemia, H = leukemia, I = icterus, M = intestinal infection  
MANN and

Case No.	Reacting types														
	1	2	3	4	5	6a	6m	7	8	9	10	11	12	13	14
53 B				c					+					+	
54 B								+			(c)				
55 B													+		
56 B				+			+	+		+				+	
57 B											(c)				
58 B				++						+	(c)				
59 B	+					+	++	++	++	+	+		++	++	
60 B			(c)		++	(c)		++	(c)		+				c
61 C															
62 C		c+								c			++		
63 C	(c)			++		+		++		++	c+	c+		(c)	(c)
64 C									+						
65 C										(c)					
66 F															
67 G		c+									c	+		+	
68 A		(c)													(c)
69 A										(c)	c				
70 A															
71 A									+						
72 A				+			+	++		+				+	
73 A								+	c+						c
74 A					+			+			+				
75 D			(c)								(c)				
76 D												+			
77 E		c		+		+			+						
78 E															
79 H								+	+						
80 M		+													
81 M	+					+		++	+						(c)
82 I	++								c+	+			+		

oil. The temperature did not rise after these injections but no more were given because of an increase in the asthma. On October 30 status asthmaticus with circulatory collapse developed. The next day the temperature rose to 38 C. and remained around 38.5 C. during the following week. At the same time, the white blood corpuscles rose from a normal level to between 11,000 and 15,000 and the sedimentation rate, which had been only slightly elevated, rose, reaching a maximum of 46 mm. in one hour on November 4. Pneumonia was

swelling reaction (upper half) and a negative reaction (lower half) types 1 to 32 (Cooper):

B = pneumonia, C = salmonella diseases, D = poliomyelitis, E = cancer, F = (not salmonella). Types 6 a and 6 m correspond to types 6 b and 6 a (KAUFF-co-workers).

of pneumococci														
15	16	17	18	19	20	21	22	23	24	27	28	29	31	32
c	c (c)+ c	+++	+	+				c ++	(c)	c++ c++ c++ c++ c++	c+ (c) (c) (c) (c)	+		
c	(c)+ c+		(c)			++				c++ c++ c++ c++ c++	c+ (c) (c) (c) (c)			
(c)	c++ (c)+ (c) (c) c++ + (c) (c)+ c+ (c)	++ + + + + + + + +	+ + + + + + + + +	+ + + + + + + + +	c + (c) + (c) ++			+++ ++ + + + + + + +	c++ + + + + + + + +	c++ c++ c++ c++ c++ c++ c++ c++ c++	(c) (c) (c) (c) (c) (c) (c) (c) (c)	+++ + (c) + + + + + +	++	
	+	++ ++	(c)		(c)	+				++	(c)	(c) (c)	c+	
++	++ +++	++ +	+	+		++								
	+	++		+				+				+		
	+	++	(c) (c)	++ ++	+									
(c)	+	+	++	++	+	+		+					+	

suspected but no signs of it could be observed roentgenographically. After treatment with oxygen and powerful cardiac and vascular stimulation, on November 5 rapid improvement set in and the patient was discharged from the hospital ten days later. On November 29 he returned with a severe recurrence of his asthma.

The sulphur did not cause the formation of nonspecific capsular swelling substance, probably because too small doses were given. On the other hand, the fever of obscure origin occurring from October 30 to

Table 9.— The occurrence of nonspecific capsular swelling substance and other substances reacting with pneumococci without being immune antibodies in sera from cases of experimentally produced nonspecific capsular swelling substance.

Date	Case 83										
	Sulfosin injections	Reacting types of pneumococci									
		2	14	16	27	28					
31/10	1 ml.	(c)+	(c)	0	0	(c)++					
1/11	2 ml.	(c)+	(c)	+	0	+++					
2/11		(c)+	(c)	0	1	(c)++					
3/11		(c)+	(c)	(c)+++	4	c+++					
4/11											
5/11											
6/11	3 ml.	c	c	(c)+++	0	0					
7/11											
8/11		(c)	(c)	(c)+	1	0					
9/11											
10/11											
11/11											
12/11		(c)	(c)	(c)++	1	(c)+					
13/11		(c)	(c)	0	0	0					
Date	Case 84										
	Allergol injections	6a	6m	9	10	15	16	17	18	27	28
20/10	0.2 ml.	(c)+	c+	c	++	c	c+++	0	c	2	++
21/10		(c)	c+	+	+	c	c+	++	(c)+	0	++
22/10	0.5 ml.	(c)+	c	c	+	c	c+++	+	c+	2	+
23/10		0	c+	+	0	(c)	c++	0	c+	+	+
24/10		++	c	+	0	c	c++	+	c+	0	+
25/10	0.8 ml.										
26/10		c+	c+	0	0	c	(c)+	+	c	1	+
27/10		c+	c++	0	+	c	c+	0	c	1	+
28/10											
29/10											
30/10		+	c	0	0	(c)	(c)+	++	(c)	8	c++
31/10											
1/11											
2/11		(c)+	c+	0	0	c+	(c)+	0	c	4	c+
3/11											
4/11											
5/11											
6/11		+	c+	0	0	c++	(c)+	0	+	8	c+++
XX											
13/11		0	0	0	0	c+	(c)++	0	+	1	+
XX											
29/11		+	c	0	0	c	0	0	0	0	0

Date	Case 85						
	Sulfosin injections	10	16	18	23	27	28
12/11	2 ml.	0	0	0	0	0	+
13/11		0	0	0	0	0	(c)+
14/11		0	c+	0	c	16	(c)+
15/11		(c)	c++	(c)	c+	32	c+
16/11	3 ml.	0	(c)	0	0	8	(c)+
17/11		0	(c)	0	0	8	(c)+
18/11		(c)	c+	(c)	c	32	c+
19/11		c	c	0	c	16	c+
20/11		(c)	(c)	0	(c)	4	(c)+
21/11		(c)	(c)	0	0	2	(c)
22/11		0	0	0	0	2	0
23/11		0	0	0	0	0	0

November 5 was accompanied by a rise in the content of the substance, manifested in an increased titer for type 27 and capsular swelling of type 28. Normal antibodies against both type 16 and type 28 seem to have occurred, for positive reactions with these types were obtained in all the specimens except for the one of November 29. For the rest, normal antibodies against several pneumococcal types were present, there being specially intense reactions in the case of types 6m, 15 and 18. The amount of normal antibodies seems to have been larger directly after admission to the hospital and showed no definite relation to the fever and condition in other respects.

Case 85 was one of a 45-year-old mentally diseased woman treated in the psychiatric ward of St. Erik's Hospital since 1933. Her somatic condition showed nothing of note. She reacted powerfully to the Sulfosin injections, showing a type 27 titer of up to 32. Type 16 reacted largely parallel to type 27 and the same was true of type 28 which, however, probably reacted with weak normal antibodies too. It is particularly interesting that types 10, 18 and 23 only reacted with sera with a high content of nonspecific capsular swelling substance.

### The Effect of Calcium on the Reaction Between Pneumococci and Nonspecific Capsular Swelling Substance and Between Pneumococci and Normal and Immune Antibodies.

After Abernethy, MacLeod and Avery had shown that the reaction between the C-polysaccharide of pneumococci and "acute phase" serum did not take place if the calcium ions were removed

from the serum, Carlens examined nonspecific capsular swelling from this viewpoint and found that it did not occur if the calcium was precipitated.

I have verified Carlens' observations through experiments of my own. I added 0.2 per cent potassium oxalate to serum (0.02 Ml. of a 10 per cent solution per milliliter of serum) or used oxalate cloth when plasma was desired. As appears from the following tables this procedure caused the disappearance of capsular swelling. The same results were obtained when serum was dialyzed against a phosphate buffer as is shown by the electrophoretic experiments described later on. On recalcification, the swelling re-appeared, which would indicate that the presence of calcium must be necessary for its development.

Table 10 shows the observations before and after the addition of oxalate in sera containing normal antibodies in addition to nonspecific capsular swelling substance and immune antibodies in one case.

Apart from a few exceptions, types 16, 27 and 28 ceased to react after the oxalate was added and thus their reactions must have consisted of nonspecific capsular swelling. The exceptions occurred in case 86 for type 16 and to a still greater degree in case 84, where the reaction persisted despite the presence of oxalate, and in case 87 for types 27 and 28 where the swelling was also not affected by the addition of oxalate. The normal antibodies reacted well even in calcium-free serum, and consequently the reactive substances against type 16 in cases 84 and 86 and against types 27 and 28 in case 87 must have been comprised, in part at least, of normal antibodies. The type 10 and type 23 reactions in case 85 were greatly influenced by the addition of oxalate, but weak agglutination persisted in the calcium-free serum. The specific agglutinins against type 3 in case 86 reacted independently of the presence of calcium.

In order to throw further light on the question whether specific antibodies against type 27 could appear at the same time as nonspecific capsular swelling substance, one subject was vaccinated with killed type 27 pneumococci. Table 11 shows this experiment.

*Case 92*, Miss G. P., aged 45, undergoing treatment for obesity at the hospital, offered herself voluntarily for the experiment. The vaccine had a bacterial density of 16 and was suspended in physiologic salt solution to which 0.1 per cent commercial formol was added. It was

Table 10. — The effect on the nonspecific capsular swelling substance and on normal and immune antibodies of adding potassium oxalate to serum.

Untr. = untreated serum, Ox. = oxalated serum.

Case	Kind of serum	Reacting types of pneumococci												
		2	3	4	6m	10	14	15	16	17	18	23	24	27
83 . . . . .	Untr. Ox.	(c)÷ (c)÷					(c) (c)		(c)÷÷÷ 0					c 0
81 <sup>21</sup> / <sub>10</sub> . . . . .	Untr. Ox.			e e				c c	(c)÷ (c)÷		c c			(c)÷ 0
82 <sup>21</sup> / <sub>10</sub> . . . . .	Untr. Ox.			e÷÷ e				c c	e÷ (c)÷		c (c)			(c) 0
85 . . . . .	Untr. Ox.					(c) ÷			e÷ 0		(c) (c)	e ÷		c 0
86 <sup>4</sup> / <sub>12</sub> . . . . .	Untr. Ox.		0						c ÷	÷÷÷ ÷÷÷				c 0
87 <sup>4</sup> / <sub>12</sub> . . . . .	Untr. Ox.		e÷ e÷						e÷ ÷	÷÷÷ ÷÷÷				0 0
87 . . . . .	Untr. Ox.			e (c)÷				c ÷	e 0			e c		c c
88 . . . . .	Untr. Ox.								(c)÷ 0	÷÷÷ ÷÷÷				c 0
89 . . . . .	Untr. Ox.								e 0					c÷ 0
90 . . . . .	Untr. Ox.								e÷ 0					c÷ 0
91 . . . . .	Untr. Ox.								e 0					c÷ 0

**Table 11. — Vaccination of 45-year-old woman (case 92) with pneumococcic vaccine type 27.**

The reactive substances against *Pneumococcus* types 16, 27 and 28 were determined both before and after the addition of potassium oxalate.

Untr. = untreated serum, Ox. = oxalated serum.

Date	Vaccine dose	Reacting types of pneumococci				Titer	
		16		27		28	
		Untr.	Ox.	Untr.	Ox.	Untr.	Ox.
$\frac{5}{6}$	0.2 ml.						
$\frac{9}{6}$		+	0	0		0	
$\frac{11}{6}$ 8 a.m.	1 ml.	++	0	0		0	
4 p.m.		++	+	+	0	0	
$\frac{12}{6}$		1	0	2	+	1	0
$\frac{13}{6}$		++	+	2	+	1	0
$\frac{14}{6}$		+	0	+++	+	0	
XX	1 ml.						
$\frac{18}{6}$		+	0	++	+	0	
$\frac{19}{6}$		2	0	4	++	2	0
$\frac{20}{6}$		2	+	8	++	1	0
$\frac{21}{6}$		2	0	4	+++	0	
XX	1 ml.						
$\frac{23}{6}$		0		4	4	0	0
XX							
$\frac{30}{6}$		0		8	8	0	
XX							
$\frac{2}{7}$	0.5 ml.	2	0	4	4	2	0
XX							
$\frac{22}{10}$							
$\frac{23}{10}$		4	0	4	4	1	0

injected intramuscularly according to the plan of dosage given in the table. The first two injections were followed by only slight malaise and fever for a few hours, but the day after the third dose the patient suffered from severe chill and her temperature rose to 40 C. The fever persisted the next day and the patient felt weak and tired. Two days later the symptoms had disappeared altogether. The fourth and fifth injections were followed by only a short period of malaise.

It appears from the table that nonspecific capsular swelling substance occurred after all except the first injection, as can be seen most easily from the type 16 and 28 reactions. Substances sensitive to oxalate appeared the day after the injections and disappeared after a few days. Type 16 seems to have reacted to a certain extent with weak normal antibodies as well. After the

third injection the untreated serum showed a type 27 titer of 8, while the oxalated blood gave titer 1 of the 2-plus type. Thus both nonspecific capsular swelling substance and specific antibodies were present and reacted simultaneously with type 27. After a time the immune antibody titer increased and concealed the nonspecific capsular swelling substance, the presence of which was manifested, however, in the simultaneously occurring type 16 and 28 reactions.

Still further experiments were done in order to examine the significance of calcium ions to nonspecific capsular swelling. Specimens were taken from a number of patients with pneumonia partly as ordinary coagulated blood samples and partly as heparinized and oxalated blood. The oxalated blood was recalcified whereupon the fibrin was precipitated. The serum or plasma was examined for nonspecific capsular swelling substance in the ordinary way and the results appear from table 12.

Table 12. — The occurrence of nonspecific capsular swelling substance in untreated serum, heparinized serum, oxalated serum and recalcified oxalated serum, tested with *Pneumococcus* type 27.

S e r u m	C u c. N o.			T i t e r	
	93	94	95	96	97
Untreated . . . . .	4	2	16	16	32
Heparinized . . . . .	4	2	16	16	16
Oxalated . . . . .	0	0	0	0	0
Recalcified oxalated . . . .	4	1	16	4	16

Table 12 further supports the observation that the presence of calcium ions is necessary for nonspecific capsular swelling. An anticoagulant such as heparin is of no importance.

### Absorption Tests.

The following method was used for the absorption experiments. One milliliter of a bacterial suspension of fixed density was centrifuged, and the precipitate suspended in one milliliter of the serum to be absorbed. One hour at room temperature was allowed for the binding.

The experiments were done firstly with pneumococcic types 27 and 28. Secondly, bacteria like *Pneumococcus* types 1 and 18,



alpha and beta streptococci and Esch. coli were tested for their power to bind nonspecific capsular swelling substance. For control purposes kaolin was used as a nonspecific absorbent. A bacterial density twice as large as the titer of the substance was used for the absorption with types 27 and 28 and one ten to one-hundred times as great for the absorption with the other bacteria. Table 13 takes up these experiments.

Table 13. — Absorption of four sera with different contents of nonspecific capsular swelling substance with different absorbents.

A27, A28, A1, A18, A $\alpha$ , A $\beta$  and Acoli denote sera after absorption with bacterial suspensions of *Pneumococcus* type 27, type 28, type 1, type 18, alpha streptococci, beta streptococci and *Escherichia coli*. A kaolin denotes sera after absorption with kaolin in concentrations with about the same optic density as the corresponding bacterial suspensions.

Serum from case	Original titer	Bacterial density used in absorption	Titer after absorption, tested against <i>Pneumococcus</i> type 27							
			A27	A28	A1	A18	A $\alpha$	A $\beta$	A coli	A kaolin
98	8	16	0	0	4					
		80			1					8
		800			0					
99	16	36	0	0	8	4	8	16	16	16
		160			2	2	4	8	8	16
		1600			0	0	0	2	0	16
100	8	16	0	0						
		80			1	4	4	8	8	8
		800			0	0	0	1	4	4
101	4	8	0	0						
		40			1	2	2	2	4	4
		400			0	0	0	0	2	4

In order better to judge the results of these experiments, the absorption of immune antibodies under the same conditions was examined. In one case serum was diluted with physiologic salt solution, so as to make the immune antibody titer correspond to those of the nonspecific capsular swelling substance. The same method was used otherwise. Table 14 shows the results of these investigations.

Table 13 shows that the nonspecific capsular swelling substance was easily absorbed by *Pneumococcus* types 27 and 28, but the effect of the other bacteria tested was more difficult to estimate. They showed absorption when the greatest bacterial concentra-

Table 14. — Absorption experiments with immune sera against pneumococci of types 1 and 5 with the same bacterial absorbents as in the foregoing experiment.

Serum from	P. type	Dilution	Original titer	Bact. density used in absorption	Titer after absorption, tested against <i>Pneumococcus</i> type 1 or 5						
					A27	A28	A1	A18	A $\alpha$	A $\beta$	A coli
Case 102	1	1/4	16	16 1600	4	8	0	16	8	8	8
Case 103	1	0	16	16 1600	16	8	0	8	8	8	16
Vacc. <sup>1</sup>	5	0	16	1600	8	16	16	16	16	16	16

<sup>1</sup> Mixed serum from military recruits vaccinated against *Pneumococcus* type 5.

tion was used and this was especially true of types 1 and 18, while kaolin caused no absorption. Table 14 shows that specific antibodies were but little influenced by even large amounts of heterologous bacteria. Whether this difference was due to the nonspecific capsular swelling substance being more unstable than immune antibodies, is not possible to say.

Experiments were also done to test the sensitivity of normal antibodies to absorption with homologous and heterologous types of pneumococci. Bacterial density 16 was used except in the cases where the titer of one of the antibodies was higher than 16, in which case a corresponding bacterial titer was used. The experiments in table 15 and 16 cover the absorption of both normal antibodies and nonspecific capsular swelling substance.

As in the case of tables 13 and 14, it must be kept in mind when judging the results that the antibodies had a low concentration. It appears, however, that normal antibodies were absorbed specifically, but to what degree heterologous absorption occurred cannot be judged from this material. The immune antibodies against type 3 in case 86 were absorbed strictly specifically.

The absorption tests cast further light upon the previous oxalate experiments. They show that the type 16 reaction in case 84 had the character of a normal antibody reaction and further strengthen the suspicion that type 23 reacted with nonspecific capsular swelling substance in case 85, as the type 23 reaction disappeared after absorption with type 16, 27 and 28. In case 87 there appeared to be both nonspecific capsular swelling substance, which was absorbed by type 27 causing the type 16

Table 15. — Absorption of normal antibodies against pneumococci compared with the absorption of nonspecific capsular swelling substance.

Serum from case	P. type tested	Bact. density used in absorption	Original titer	Titer after absorption											
				A2	A3	A6m	A14	A15	A16	A17	A18	A23	A27	A28	
83	2	16	1	0			1		1				1		
	14	»	1	1			9		1				1		
	16	»	4	4			4		0				0		
	27	»	4	4			4		4				0		
	28	»	4	2			4		0				0		
84	6m	»	8			0		2	1		4		1		
	15	»	4			2		0	2		2		1		
	16	»	4			1		1	0		1		1		
	18	»	1			1		1	0		0		1		
	27	»	1			0		0	0		0		0		
85	16	32	16						0			8	0		
	23	»	4						0			0	0		
	27	»	32						4			8	0		
	28	»	8						0			4	0		
86 <sup>4</sup> / <sub>10</sub>	16	16	16						0	8			0		
	17	»	4						4	0			4		
	27	»	16						4	8			0		
	28	»	16						4	8			0		
86 <sup>21</sup> / <sub>10</sub>	3 <sup>1</sup>	»	16		0				16	16			16		
	16	»	16		8				0	8			8	1	
	17	»	16		16				8	0			16		
87	16	64	4						0			4	0		
	23	»	16						18			0	8		
	27	»	64						6			32	0	16	
	28	»	8						0			2	0	0	
88	16	16	4						0	4			0		
	17	»	16						8	0			8		
	27	»	16						4	8			0		

<sup>1</sup> Immune antibodies.

and 28 reactions to disappear, and normal antibodies against type 27, which withstood absorption with types 16 and 28. However, absorption does not give any definite explanation for the type 16 reaction in case 86; in the specimen of October 4 the reactive substance was absorbed by both type 27 and type 28, but heterologous absorption did not occur in the specimen of October 24.

The nonspecific capsular swelling substance was absorbed by

Table 16. — Absorption of nonspecific capsular swelling substance with pneumococci of types 16, 27, and 28.

Serum from case	P. type tested	Bact. density used in absorption	Original titer	Titer after absorption		
				A16	A27	A28
89	16	16	4	0	0	0
	27	»	8	4	0	0
	28	»	8	0	0	0
	27	64		0		
90	16	16	8	0	0	0
	27	»	8	8	0	0
	28	»	8	0	0	0
	27	64		4		
91	16	16	8	0	0	0
	27	»	8	2	0	0
	28	»	4	0	0	0
	27	64		0		
92	16	16	2	0	0	0
	27	»	4	4	0	4
	28	»	2	0	0	0
	27	64		4		4

types 27 and 28 in case 83, 85 and 86 in table 15 and in cases 89, 90 and 91 in table 16. Type 16 absorbed nonspecific capsular swelling substance reciprocally in regard to type 28 (cases 83, 85 and 87 in table 15 and cases 89, 90 and 91 in table 16) with one exception, case 86, where the type 28 reaction remained even after the absorption. The nonspecific type 27 reaction was not as easily affected by absorption with type 16, as is seen specially from cases 89, 90 and 91. Bacterial density 16 of type 16 absorbed the reactive substance for types 16 and 28, while the type 27 titer only decreased. Density 64 of type 16 was sufficient, however, to cause the disappearance of the type 27 reaction as well in cases 89 and 91.

In case 92 in table 16 the absorption showed that nonspecific capsular swelling substance occurred together with immune antibodies against type 27 in the serum. The blood specimen was taken three days after the fourth injection of vaccine (table 11). Density 16 of type 27 absorbed the nonspecific capsular swelling substance, causing the disappearance of the type 16 and type 28 reactions, but not even density 64 of types 16 and 28 could decrease the content of immune antibodies.

## Attempts to Estimate the Antibacterial Capacity of Nonspecific Capsular Swelling Substance.

The antibacterial capacity of nonspecific capsular swelling substance was tested with pneumococci of types 27 and 28. *Pneumococcus* type 1 was used for control experiments.

The pneumococcic strains used in these experiments were kept dried in a cold room. One stock tube was consumed for each experiment. In the morning the tubes containing the dried culture were filled with broth (meat extract broth plus 10 per cent ascitic fluid and 0.1 per cent dextrose). The culture was allowed to grow during the day, and then 0.5 Ml. of a suitable culture dilution was injected intraperitoneally into a white mouse. The next day, when the mouse was dying or dead, broth cultures were made from the cardiac blood. After the bacteria had grown for six to eight hours at 37 C., the main experiment was done. The cultures were diluted according to the virulence of the strain, and undiluted serum was mixed in the test tube with equal parts of culture dilution. After half an hour, 0.4 Ml. of these mixtures were injected intraperitoneally into mice weighing between 16 and 18 grams. The mice were kept under observation for four days. The animals used all came from the mice stocks of the laboratory. <sup>1</sup>

The sera tested showed a substance titer of 8 or 16. Control sera were taken from uncomplicated cases of hypertension. Table 17 gives the results of these experiments.

The nonspecific capsular swelling substance did not reduce the lethality in infections with pneumococci of types 1, 27 and 28 but it appeared to give a certain amount of temporary protection against infections with types 27 and 28. The number of animals dying during the first experimental day in the type 27 experiments was  $0 \pm 1$  for the sera containing the substance and  $21 \pm 2.96$  for the control sera, and the corresponding figures for the type 28 experiments were  $3 \pm 1.58$  and  $11 \pm 2.07$ . The differences were  $21 \pm 3.14$  and  $8 \pm 2.61$ , respectively, thus proving statistically that the mortality during the first day in the case of both type 27 and type 28 infections was lower for sera containing nonspecific capsular swelling substance than for control sera. The protective effect of the substance was only noticeable in the experimental series where such a large infecting dose was given that the control animals died within twenty-four hours. The same was not observed in the cases of type 1 infection where, on the contrary, the control sera seemed to give more protection than the sera containing the substance.

## Fractionation of Sera Containing Nonspecific Capsular Swelling Substance.

As mentioned previously, MacLeod and Avery demonstrated, by means of the salting out process with sodium sulphate that the reactive substance in "acute phase" serum occurred in the albumin fraction. In order to compare nonspecific capsular

swelling substance with this substance and in order to study its relationship to normal antibodies, a number of fractioning experiments were done.

Precipitation by half saturation with ammonium sulphate showed that both nonspecific capsular swelling substance and normal antibodies were contained in the globulin fraction of serum.

Serum (case 110) containing nonspecific capsular swelling substance and normal antibodies was dialyzed against distilled water for eighteen hours in a cellophane bag at 4 C. and the results are given in table 18. The precipitate obtained, euglobulin, was dissolved to its original volume in physiologic salt solution.

The table shows that the active substances were present in the euglobulin. The yield of nonspecific capsular swelling substance was about 25 per cent.

The nonspecific capsular swelling substance was also studied with electrophoresis.<sup>1</sup> The apparatus used was of the Tiselius' type with special arrangements for the separation of the different serum fractions as described by Svensson (89). A sample of serum from a patient with myocardial infarction (case 91) was kept frozen until the experiment six days later.

The separation was done with undiluted serum at pH 7.6 (160 Ml. of fifth molar solution of  $\text{Na}_2\text{HPO}_4$  and 20 Ml. of a fifth molar solution of  $\text{Na}_2\text{PO}_4$ , per liter) at a temperature of + 0.4 C. for eighteen hours. A current of 17.0 milliamperes was used the first five hours and thereafter one of 7.0 milliamperes. Fractions were taken after 515 minutes and at the end of the experiment. The separation was done under optic supervision and it was observed that the albumin peak was duplicated, two albumin fractions occurring on the positive side. From the positive side were obtained fractions albumin I, albumin I + II, albumin +  $\alpha$  and albumin +  $\alpha$  +  $\beta$  and from the negative side fractions  $\gamma$ ,  $\gamma$  +  $\beta$ ,  $\gamma$  +  $\beta$  +  $\alpha$ ? and  $\gamma$  +  $\beta$  +  $\alpha$  + albumin II. The fractions were examined for reactive substances, both as they were obtained from the electrophoresis and after dialysis against physiologic salt solution containing 0.025 per cent of calcium chloride. The dialysis was done in order to add calcium as it had been precipitated by the buffer solution used in the electrophoresis.

Table 19 shows the distribution of the nonspecific capsular swelling substance and of normal antibodies against types 18, 21 and 31 in the different protein fractions.

The table shows that there was no nonspecific capsular swelling substance in calcium-free serum, if one disregards the weak type 28 reactions for which no explanation is forthcoming. Nonspecific capsular swelling substance could be demonstrated in

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<sup>1</sup> The experiments were done by Dr. Harry Svensson at the Institute of Physical Chemistry of Uppsala University.

Table 17. — The antibacterial capacity of nonspecific capsular swelling  
1, 27 and 28 compared with the  
NCS = serum containing nonspecific capsular swelling

Serum	P. type	No. of animals per dilution	Culture dilution	Results						Culture dilution
				Dead					Surviving	
				1st day	2nd day	3rd day	4th day	Total		
									Protection test	
NCS										
104	1	6	$10^{-3}$	5	1			6	0	$10^{-3}$
105	1	6	"	4	2			6	0	"
106	1	6	"	6				6	0	"
CS										
107	1	6	"		6			6	0	"
108	1	6	"		6			6	0	"
109	1	6	"		6			6	0	"
Total										
NCS	1	18	$10^{-3}$	15	3			18	0	$10^{-3}$
CS	1	18	"		18			18	0	"
Vir	1	8	$10^{-6}$		8			8	0	$10^{-7}$
									Protection test	
NCS										
104	27	18	$10^{-2}$		11	4		15	3	$10^{-3}$
105	27	18	"		13	1	3	17	1	"
CS										
107	27	18	"	13	5			18		"
108	27	18	"	8	7			15	3	"
Total										
NCS	27	36	$10^{-2}$		24	5	3	32	4	$10^{-3}$
CS	27	36	"	21	12			33	3	"
Vir	27	4	$10^{-2}$	2	2			4	0	$10^{-3}$
									Protection test	
NCS										
104	28	6	$10^{-1}$	3	2	1		6	0	$10^{-2}$
110	28	12	"		7	4	1	12	0	"
CS										
107	28	6	"	3	3			6	0	"
108	28	12	"	8	4			12	0	"
Total										
NCS	28	18	$10^{-1}$	3	9	5	1	18	0	$10^{-2}$
CS	28	18	"	11	7			18	0	"
Vir	28	9	$10^{-1}$	5	4			9	0	$10^{-2}$

### Clinical Symptoms.

In a large proportion of the cases *prodromal symptoms* were observed before the exanthema appeared. In the group with mucous membrane affections prodromes occurred in one third of the cases, in the form of »symptoms of a cold», feverishness, indisposition, cough, that is to say, uncharacteristic symptoms. 3 of the 28 had vomitings, 2 had pains in the joints, 1 had dysphagia. The prodromes lasted from 2 or 3 up to 8 days, occasionally longer.

In the group with frank E. m., without symptoms from mucous membranes, the situation was about the same. Only two patients complained of pains in the joints before the exanthema appeared.

In the group with combined E. m. and E. n., on the other hand, prodromes appeared in *twice as many cases*, namely, in two thirds of the patients, and here they were of a somewhat different character: most often dysphagia, *pains in the joints*, as well as feverishness, coughing, fatigue — symptoms which are also met with in the prodromal stage of the frank E. nodosum.

The diagnoses on which the patients were admitted to hospital give a little hint respecting the clinical picture in the initial stage. A large number were admitted for acute rheumatism or rheumatic fever, some few for fever of unknown cause, rather many for erythema nodosum, some for meningitis or poliomyelitis, besides many different forms of exanthema.

As regards the relationship between exanthema and mucous membrane affection, it has here not been possible to find any characteristic *sequence* in the symptoms. The general experience seems to have been that exanthema and mucous membrane symptoms appeared at the same time. In some few cases the stomatitis came some days before the exanthema, but the reverse order of appearance has also been observed. The affection of the eyes was occasionally not noted until somewhat later on in the course of the illness. We get a distinct impression that, clinically regarded, exanthema and mucous membrane symptoms are *co-ordinate phenomena*, and that we cannot speak of exanthema as a complication in stomatitis, or *vice versa*.

Affection of the mucous membrane of the nose, often with epistaxis, was noted in 12 patients, suppurative otitis media in 3, pharyngitis in 20, laryngitis in 6, including one patient with a large laryngeal ulcer, gastritis in 3, enteritis in 1 and affection of



Table 18. — Dialysis of serum containing nonspecific capsular swelling substance and normal antibodies against pneumococci of types 10 and 18.

Case 110	N.C.S.		Normal antibodies	
	type 27	type 16	type 10	type 18
Untreated serum . . . . .	8	c+	(c)	(c)
Water-insoluble fraction . . . . .	2	c+	(c)	c+
Serum minus water-insoluble fraction .	0	0	0	0

Table 19. — Electrophoretic fractionation of human serum containing nonspecific capsular swelling substance and normal antibodies against pneumococci of types 18, 21 and 31, showing the content of reactive substances before and after recalcification.

Before fractionation the serum showed a titer of 8 for type 16, of 2 for type 18 and 21 and of 8 for type 27, 28 and 31.

Case 91 Fractions	Reacting types of pneumococci										Titer	
	Before dialysis						After dialysis					
	16	18	21	27	28	31	16	18	21	27	28	31
Positive side												
Albumin I . .	0	0	0	0	0	0	0	0	0	0	0	0
Albumin I+II	0	0	0	0	0	0	0	0	0	0	0	0
Albumin+ $\alpha$ .	0	0	0	0	0	0	1:(c)	0	0	1:(c)	1:(c)	0
Albumin+ $\alpha$ + $\beta$	0	0	0	0	1:(c)	0	4	2	1	4	4	0
Negative side												
$\gamma$ . . . . .	0	1:+	1:+	0	1:(c)	1:(c)	2	0	1:+	2	2	1:(c) +
$\gamma$ + $\beta$ . . . .	0	2	1:(c) +	0	1:(c)	4	2	2	1:+	4	4	4
$\gamma$ + $\beta$ + $\alpha$ ? . . .	0	2	2	0	1:(c)	4	4	2	2	4	4	4
$\gamma$ + $\beta$ + $\alpha$ +alb. II	0	1	2	0	1:(c)	8	8	1	1	8	4	4

<sup>1</sup> 1:(c) denotes titer 1 with capsular swelling of type (c).

recalcified serum in all fractions of the negative side, and on the positive side in the albumin +  $\alpha$  +  $\beta$  fraction together with a trace in the albumin +  $\alpha$  fraction. The original titer for the substance was 8, and half the activity occurred in the albumin +  $\alpha$  +  $\beta$ ,  $\gamma$  +  $\beta$  +  $\alpha$ ? and  $\gamma$  +  $\beta$  fractions, with the exception of the type 16 reaction which only had a titer of 2 in the  $\gamma$  +  $\beta$  fraction. The  $\gamma$  fraction reacted with a titer of 2 with both type 16 and types 27 and 28.

Although one has to be cautious about conclusions because of the weak serologic reactions, the experiment would indicate that the nonspecific capsular swelling substance moved faster than the  $\gamma$  globulin, as it occurred on the positive side in a  $\gamma$ -free fraction, but slower than the albumin, as it was not present in the albumin fraction.

The reactive substance against pneumococci of types 18, 21 and 31 occurred in calcium-free serum only in fractions from the negative side and therefore moved slower than nonspecific capsular swelling substance. In recalcified serum reactive substances against types 18 and 21 were also found in the positive side's albumin +  $\alpha$  +  $\beta$  fraction, which might indicate that these types reacted with nonspecific capsular swelling substance in this fraction.

Another electrophoretic experiment was done with a specimen of serum from a patient in the psychiatric ward of St. Erik's Hospital (case 111). Nonspecific capsular swelling substance was produced by the subcutaneous injection of 3 Ml. of Sulfosin on July 30, 1942. The blood specimen was taken two days later and the serum then kept frozen.

The experiment was done with undiluted serum on August 11 (experiment 23) and again on August 20 (experiment 27) in a standard Tiselius apparatus.

The pH was 7.6 and the temperature + 0.5 C. In experiment 23 current of 19.4 milliamperes was used during the first four hours and afterwards one of 2.2 milliamperes, and in experiment 27 one of 20.9 milliamperes was used throughout. Experiment 23 was allowed to proceed for twenty hours. At the end fractions albumin +  $\alpha$  +  $\beta$  and albumin +  $\alpha$  +  $\beta$  +  $\gamma$  were taken under optic supervision from the positive side and fractions  $\gamma$  +  $\beta_1$  +  $\beta_2$  and  $\gamma$  +  $\beta$  +  $\alpha$  + albumin from the negative side. Experiment 27 was kept up for 248 minutes. At the end, fractions albumin +  $\alpha$  and albumin +  $\alpha$  +  $\beta$  +  $\gamma$  were isolated from the positive side and fractions  $\gamma$  +  $\beta$  +  $\alpha$  and  $\gamma$  +  $\beta$  +  $\alpha$  + albumin from the negative side.

The serum contained nonspecific capsular swelling substance but not normal antibodies. Table 20 shows the distribution of the nonspecific capsular swelling substance over the different fractions.

In experiment 23 the top fraction on the positive side, albumin +  $\alpha$  +  $\beta$ , contained reactive substance and the top fraction on the negative side none. In experiment 27 the top fraction on the positive side, albumin +  $\alpha$ , contained weak reactive substance, while the negative side's  $\gamma$  +  $\beta$  +  $\alpha$  fraction still contained none.

The concentrations of the  $\alpha$  and  $\beta$  globulins in the top fractions compared with the content of substance are seen in table 21.

**Table 20. — Electrophoretic fractionation of human serum containing nonspecific capsular swelling substance, and taken two days after the subcutaneous injection of Sulfosin.**

Before the fractionation, the serum showed a titer of 16 for types 16 and 27 and of 32 for type 32.

Case 111 Experiment 23 Fractions	Reacting types of pneumococci Titer					
	Before dialysis			After dialysis		
	16	27	28	16	27	28
Positive side						
Albumin+ $\alpha$ + $\beta$ . . . . .	0	0	0	2	2	1
Albumin+ $\alpha$ + $\beta$ + $\gamma$ . . . . .	0	0	0	8	4	4
Negative side						
$\gamma$ + $\beta_1$ + $\beta_2$ . . . . .	0	0	0	0	0	0
$\gamma$ + $\beta$ + $\alpha$ +alb. . . . .	0	0	0	16	8	4
Experiment 27 Fractions						
Positive side						
Albumin+ $\alpha$ . . . . .	0	0	0	1	1	1
Albumin+ $\alpha$ + $\beta$ + $\gamma$ . . . . .	0	0	0	8	4	4
Negative side						
$\gamma$ + $\beta$ + $\alpha$ . . . . .	0	0	0	0	0	0
$\gamma$ + $\beta$ + $\alpha$ +alb. . . . .	0	0	0	16	8	4

**Table 21. — The content of nonspecific capsular swelling substance in the top fractions in experiments 23 and 27 compared with the concentrations of  $\alpha$  and  $\beta$  globulin in the same fractions, in per cent of the concentrations in the serum.**

Experiment 23 Top fractions	Concentrations in per cent				
	N.C.S.	$\alpha$	$\beta_1$	$\beta_2$	$\beta$
Albumin+ $\alpha$ + $\beta$ . . . . .	25	56	—	—	22
$\gamma$ + $\beta_1$ + $\beta_2$ . . . . .	0	0	7	18	25
Experiment 27 Top fractions					
Albumin+ $\alpha$ . . . . .	12.5	20	—	—	< 1
$\gamma$ + $\beta$ + $\alpha$ . . . . .	0	< 9	24	36	60

Nonspecific capsular swelling substance occurred in fractions containing  $\alpha$  in more than 12.5 per cent. A lower content of the substance is not titratable when the original titer is 8. The substance was present in  $\beta$ -free fractions, albumin +  $\alpha$ , but was lacking in fractions with a high  $\beta$  content,  $\gamma + \beta + \alpha$ .

Comparison of the three electrophoretic experiments shows that the results of the first and the second two do not entirely agree. The fraction albumin +  $\alpha + \beta$  contained active substance in the first experiment and experiment 23, and likewise fraction albumin +  $\alpha$  showed a trace of activity in the first experiment and experiment 27. In the first experiment, all the fractions of the negative side contained active substance, but in experiments 23 and 27 it was lacking in both  $\gamma + \beta_1 + \beta_2$  and  $\gamma + \beta + \alpha$ . The results of experiments 23 and 27 would indicate that the active substance was present in the  $\alpha$  fraction and that the concentration of  $\alpha$  in the  $\gamma + \beta + \alpha$  fraction in experiment 27 was too low to cause the capsular swelling reaction.

In summary, the electrophoretic experiments show that the nonspecific capsular swelling substance belongs to the globulin fraction of the serum and moves faster than the  $\gamma$  globulin, but they reveal nothing definite about their relationship to the  $\beta$  and  $\alpha$  globulins. The normal antibodies which are found in calcium-free serum probably belong to the  $\gamma$  globulin. Further research is necessary to clear up these problems.

### Attempts to Produce Nonspecific Capsular Swelling Substance Experimentally in Man.

As already reported, Carlens examined the serum of patients given injections of colloidal sulphur for desensitization and found nonspecific capsular swelling substance in all the cases where good fever reactions were produced.

I made similar experiments, using the sulphur preparation Sulfosin (Leo) which contains 10 mg. of sublimed sulphur per milliliter of oil. The experimental subjects were patients in the psychiatric ward of St. Erik's Hospital and samples of blood were taken during the fever therapy. Figure 1 shows the cases studied.

The subjects were all women between the ages of 30 and 50 and had been treated for several years at the hospital under the diagnosis schizophrenia. In case 85 the blood samples were taken in connection with the second and third Sulfosin injection of a

## NONSPECIFIC CAPSULAR SWELLING.

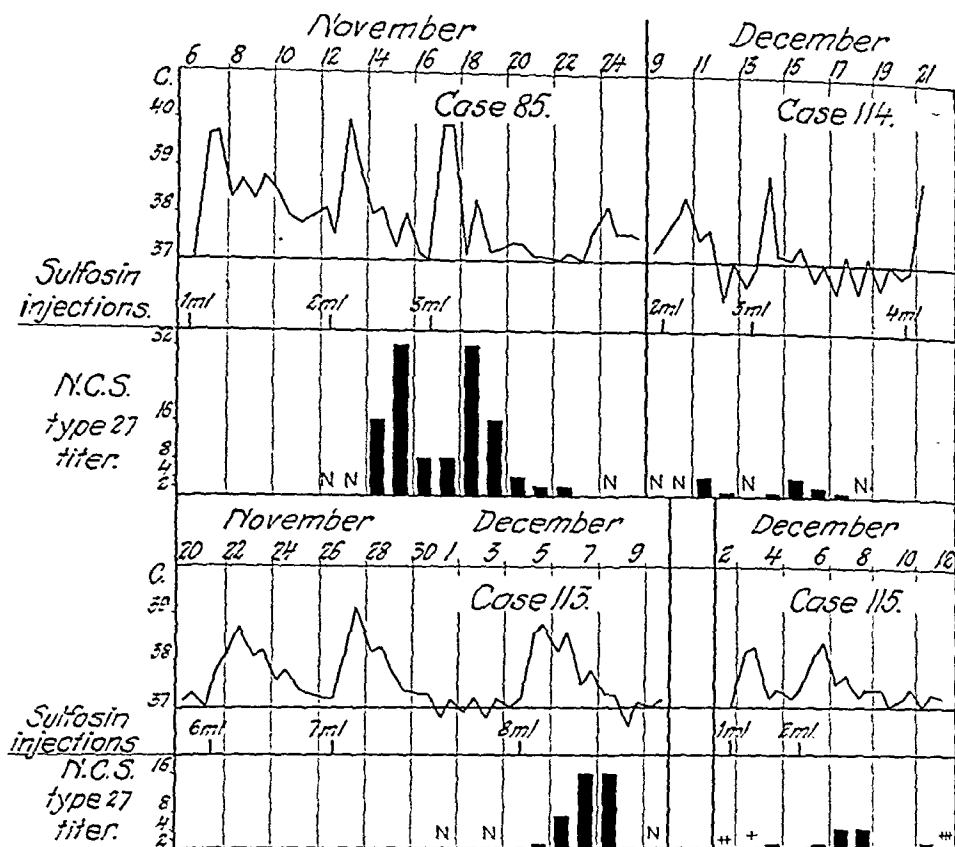


Fig. 1. Four cases, in which nonspecific capsular swelling substance (N. C. S.) had been produced by subcutaneous injections of Sulfosin (colloidal sulphur suspended in oil). N = negative.

series, in case 113 in connection with the eighth injection, and in cases 114 and 115 from the beginning of the series. The first two patients (cases 85 and 113) had received similar treatment earlier in the year; the second two patients (cases 114 and 115) had had no such treatment for several years. The experiments showed that the Sulfosin produced nonspecific capsular swelling substance in all the cases, and that the amount of the substance generally reached a maximum on the second to third day after the injection. The concentrations then decreased and the blood tests became negative within a week. The fever peaks culminated within twenty-four hours after the injection, and thus the fluctuations in body temperature did not run parallel with the changes in the concentrations of the substance. It seems, however, as if the highest content of substance was reached in the cases which showed the most violent fever reactions.

## CHAPTER 5.

### **The Occurrence of Nonspecific Capsular Swelling Substance and Normal Antibodies Against Pneumococci in Blood from the Horse, Rabbit and Guinea Pig.**

When investigating into the occurrence of nonspecific capsular swelling substance in horse, rabbit and guinea pig blood, the reactive substances found had to be divided into nonspecific capsular swelling substance and normal antibodies, as in the case of man. Substances which were not present in normal serum, but which appeared in acute stages of disease and which required the presence of calcium for their reaction with antigen to be microscopically visible, were counted as nonspecific capsular swelling substance. Normal antibodies occur in normal sera and their reaction with antigen is independent of calcium. The same method was used for the study of the animal sera as that previously used.

#### **Examination of Horse Serum.**

First, I examined the sera from healthy horses which had never been used for the production of immune serum or which had not undergone any immunization for two months before the blood was taken. The latter consisted for the most part of horses used for the production of antidiphtheric serum, never inoculated with bacteria of any kind. The results of the examination for normal antibodies appear from table 22.

The horse sera reacted with a large number of pneumococcic types with certain individual variations for the different animals. In order to try to produce capsular swelling substance, the horses Gregor, Gamin and Gråbo were inoculated several times with diphtheric toxin mixed with tapioca. A high fever developed after the injections but the sera contained about the same normal antibodies as before the injection, and no substance comparable with the nonspecific capsular swelling substance in man was encountered.

Table 22. — The occurrence of normal antibodies against pneumococci of types 1—32 in the horse.

Name of horse	Reacting types of pneumococci														
	1	2	3	4	5	6a	6m	7	8	9	10	11	12	13	14
Gamin . . . . .	+	c+		+	+	+		+	(c)	(c)+	c+	(c)+	+	c+	c
Glader . . . . .		+		+			+	+		+	+	+	+	+	(c)
Gregor . . . . .	+			+	+	+	+	+	c+	c	c+	c+	+	c+	c
Gräbo . . . . .	+	c		+	+	+	+	+	+	+	c+	c+	+	c+	+
Garibaldi . . . . .	+	c+		+	+	+	+	+	+	+	(c)+	(c)+	+	+	+
Gabriella . . . . .					+		+	+	+	(c)+	c	(c)+	+	+	(c)
Lise . . . . .	+	c+		+	+	+	+	+	+	+	c	(c)+	+	+	+
Dante . . . . .	+	+		(c)+	+	+	+	+	+	+	(c)+	(c)+	+	(c)+	c
Grip . . . . .	+			c+	+	+	+	+	+	+	c+	(c)+	+	+	+
Gotte . . . . .		(c)+			+	+	+	+	+	+		(c)+	+	+	+
Name of horse	16	17	18	19	20	21	22	23	24	25	27 titer	28	29	31	32
Gamin . . . . .	c+	+	(c)+	+	+	+	+	+	c+	+	4	(c)+	+	(c)+	+
Glader . . . . .	+	+	c+	+	+	+	+	+	+	+	1	+	c	+	+
Gregor . . . . .	+	+	+	+	+	+	+	+	+	+	4	(c)+	+	+	+
Gräbo . . . . .	c+	+	c+	+	c+	+	+	+	c+	+	4	(c)+	+	c+	+
Garibaldi . . . . .	c+	+	c+	+	(c)+	+	+	(c)+	+	+	(c)+	+	+	+	+
Gabriella . . . . .	+	+	+	+	+	+	+	+	(c)+	+	+	+	+	+	+
Lise . . . . .	c+	+	(c)+	+	c+	+	+	c+	+	+	+	+	(c)+	+	+
Dante . . . . .	c+	+	+	+	+	+	+	+	+	+	+	+	(c)+	+	+
Grip . . . . .	c+	+	+	+	(c)+	(c)+	+	(c)+	(c)	+	(c)+	+	(c)+	(c)	c+
Gotte . . . . .	c+	+	+	+	c+	(c)+	+	+	+	+	4	+	+	(c)	c+

### Examination of Rabbit Serum.

Rabbits proved to be more suitable animals for the production of capsular swelling substance. In preliminary testing of nine rabbits weighing about 2.5 Kg., no normal antibodies against pneumococci of types 1 to 32 were observed. Five of the animals were given intramuscular injections of Sulfosin in one thigh, and blood was taken through cardiac puncture almost daily thereafter. Type 16 reacted with sera from four of the five rabbits after the injection, showing capsular swelling and agglutination. Types 27 and 28 reacted much less, the former only occasionally with weak and indistinct capsular swelling, the latter more but only in sera with a relatively high titer for type 16. Nor did the other types of pneumococci react with these sera after the nonspecific stimulation. Two of these experiments, covering the reacting types, 16, 27 and 28, are shown in the upper third of figure 2.

The middle third of figure 2 shows the results of vaccinating four rabbits with intravenous injections of killed *Pneumococcus* type 3. The vaccine had a density of 100. Despite this fairly intensive immunization, specific agglutination was not observed in three of the four animals. The fourth, rabbit 1, gave a weak specific reaction on the twelfth day. On the other hand, all the animals' sera reacted with type 16 and to a certain extent with type 28 after the injections. Type 27 did not react with any of the sera.

Eight rabbits were immunized with pneumococcic type 1 vaccine of a density of 160 in order to permit a study of the behavior of nonspecific capsular swelling substance when specific antibodies were present. Four animals weighed about 2.5 Kg. and the others about 1.3 Kg. This experimental series is shown in the lower third of figure 2. Slide agglutination showed the presence of specific agglutinins the fourth day after the first injection and their concentration increased as the experiment went on. Nonspecific capsular swelling substance appeared the day after the first dose and reacted with type 16, and when there was a high type 16 titer, with type 28 as well, but never with type 27. On the third day after each dose, at the latest, the serum contained no substance but it reappeared in the older animals — the four left bars in the figure — after each new dose of vaccine, and its



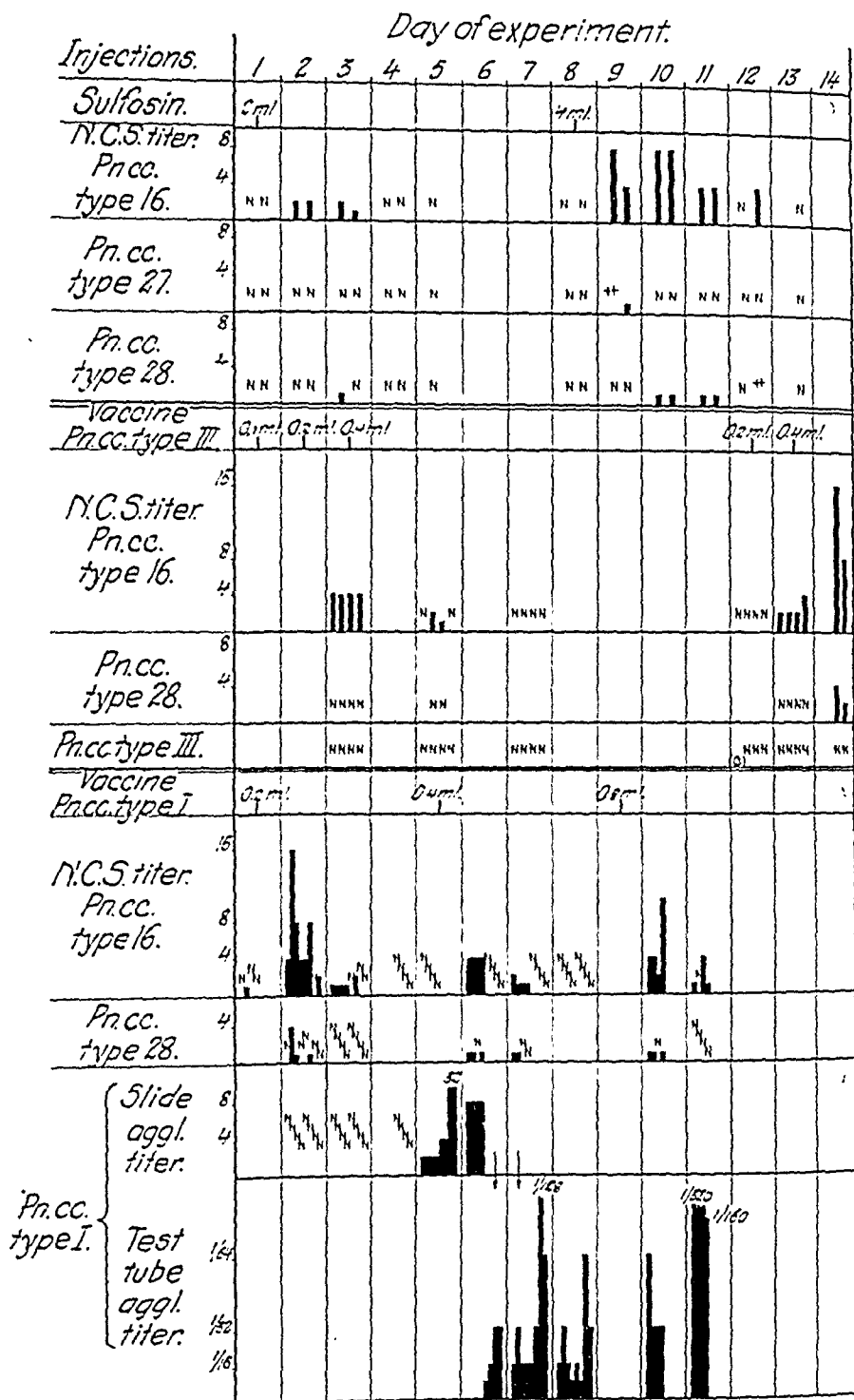


Fig. 2. Experimentally produced nonspecific capsular swelling substance (N. C. S.) and immune antibodies against pneumococci of types 1 and 3 in rabbits. Each bar and N represent one animal. N = negative.

concentration varied quite independently of that of the specific agglutinins.

The slide method of examination was complemented in a number of cases with test tube tests, and both methods confirmed that this reactive substance in rabbits had essentially the same properties as the nonspecific capsular swelling substance in man. The capsular swelling appeared first and the agglutination afterwards, and no reaction was obtained with oxalated plasma, but after recalcification it appeared again.

The absorption of the nonspecific reactive substance in rabbits by types 16, 27 and 28 was examined. The results are collected in table 23.

Table 23. — Absorption of the nonspecific capsular swelling substance in rabbit serum with pneumococci of types 16, 27 and 28.

Rabbit serum no.	P. type tested	Bact. density used in absorption	Original titer	Titer after absorption		
				A16	A27	A28
6	16	16	8	0	4	0
	16	64	"	—	0	—
5 and 8	16	16	8	0	4	0
	16	64	"	—	0	—

It is seen that the substance was easily absorbed with types 16 and 28, but less easily with type 27.

### Examination of Guinea Pig Serum.

It was also shown that guinea pig serum contains normal antibodies. Table 24, which covers this part of the investigation, includes only the types of pneumococci which reacted with serum.

Table 24. — The occurrence of normal antibodies causing capsular swelling and agglutination of pneumococci in guinea pig serum.

Results	Reacting types of pneumococci				No. of sera
	10	19	27	28	
Positive sera . . . . .	1	10	5	2	16
Type of reaction . . . . .	(c)	+++	c	c	
Highest titer . . . . .	—	< 16	16	8	

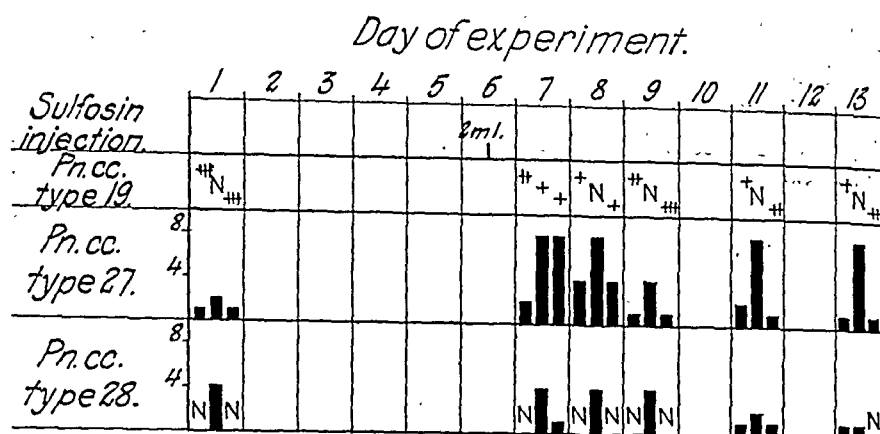


Fig. 3. Experiments with injections of Sulfosin in guinea pigs. Each bar and N represent one animal. N = negative.

The most common normal antibody in guinea pigs was directed against type 19, and the next most common one against type 27. The type 19 antibody only caused agglutination, but the type 27 antibody caused capsular swelling of maximum size, comparable with the reaction obtained with immune serum. It appeared larger than the nonspecific capsular swelling observed in human serum. Reactions with type 28 occurred in two cases and with type 10 in one case.

Experiments were also done with Sulfosin. Figure 3 illustrates experiments with three guinea pigs, the sera of which contained normal antibodies against type 27. Three other animals lacking antibodies of this kind were also given injections of Sulfosin. In the former group each guinea pig weighed 500 Gm. and in the latter group about 300 Gm. All the experiments covered types 1 to 32 but only types 19, 27 and 28 gave positive reactions and were included in the figure. The Sulfosin was injected into the muscles of the thigh and samples of blood were taken with cardiac puncture.

The antibodies present in the serum before the injections of Sulfosin remained throughout the experiment. In the animals taken up in the figure, the content of type 27 antibodies increased the first few days after the Sulfosin injection together with a slight rise in the temperature, especially in cases 2 and 3. However, in the case of animal 2, the reaction remained at a high degree even when the effect of the Sulfosin had probably subsided. The three cases which did not show any normal antibodies against

type 27 in the beginning showed no reactive substances against this type after the injections of Sulfosin. Oxalated plasma from guinea pigs gave the same reactions on the whole as serum containing calcium, and consequently the type 27 antibodies were not sensitive to calcium.

## CHAPTER 6.

### The Occurrence of Nonspecific Capsular Swelling Substance in Different Diseases.

My first paper on nonspecific capsular swelling substance dealt mainly with its occurrence in acute pneumonia and acute infections of the upper respiratory tract. The substance was found in every case of pneumonia and in cases of infection in the respiratory passages with a tendency to bacterial complication. I pointed out that it was not confined to pneumococcic infections but also occurred in infections caused by hemolytic streptococci and tubercle bacilli.

In a later paper it was shown that the substance also occurred in cases of disease where a bacterial origin could be excluded, and where the morbid process was comprised of rapidly ensuing tissue disintegration, as in acute cardiac infarction. As regards diseases of bacterial origin, the substance was also demonstrated in typhoid and paratyphoid fever, but not in virus infections.

Since these two publications, still further cases have been examined, some of the same diseases as studied before, and some of other infections and tumor diseases.

Most of the patients were treated at St. Erik's Hospital. The cases of scarlatina and the ones examined during vaccination for smallpox were taken from a Stockholm regiment. The cases of mammary cancer came from Radiumhemmet, the cases of poliomyelitis from the Epidemic Hospital in Stockholm and most of the cases of pulmonary tuberculosis from the Söderby Hospital. Sera sent to the State Bacteriologic Laboratory for Widal testing were examined in positive cases, especially when there was bacterial growth in the blood. In addition, samples of blood from occasional cases of different diseases were obtained from the Maria Hospital, the Epidemic Hospital and Norrull's Hospital.

Some cases were followed with repeated tests, in others only one or two specimens of blood were taken.

The cases are assembled in Table 25. The days the specimen were taken are grouped into weeks from the time the patient fell ill.

Table 25.— The occurrence of nonspecific capsular swelling substance in certain diseases.

D i a g n o s i s	W e e k o f d i s e a s e s e r a e x a m i n e d													
	1st week		2nd week		3rd week		4th week		>4th week		Total			
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	No.	Pos.	Neg.	
<i>Acute pneumonia . .</i>	143	8	15	3	1	0						170	159	11
<i>Septicemia . . . . .</i>	3	0	3	1	1	0			5	0		13	12	1
<i>Salmonella infections .</i>			10	1								11	10	1
<i>Scarlatina . . . . .</i>	16	30										46	16	30
<i>Pulmonary tuberculo- sis . . . . .</i>	9	1	4	2	1	3	1	2	9	22	54	23	31	
<i>Meningitis:</i>														
Bacterial . . . . .	4	0	1	0					1	0	6	6	0	
Non-bacterial . . . . .	0	4	0	1					0	1	6	0	6	
Tuberculosis . . . . .			0	1					1	0	2	1	1	
<i>Virus diseases:</i>														
Poliomyelitis . . . . .	1	12	0	4	0	1					18	1	17	
Smallpox vaccination .		1	21								22	1	21	
<i>Cardiac and vascular diseases:</i>														
Myocardial infarct . .	6	0					1	0			7	7	0	
Heart decompensation														
Chronic valvular dis- ease														
Cardiosclerosis . . . .	0	7							0	5	12	0	12	
Nephrosclerosis . . . .									0	7	7	0	7	
Deep thrombosis . . . .	0	3									3	0	3	
Emboli . . . . .	0	3									3	0	3	
<i>Tumors:</i>														
Bronchial carcinoma .									1	0	1	1	1	
Hypernephroma . . . .									1	0	1	1	1	
Cancer of the stomach										1	1			
Cancer of the prostate									0	1	1	0	1	
Sarcoma . . . . .					0	1					1	0	1	
Cancer of the breast .					0	2			0	6	8	0	8	

### Acute Pneumonia.

Sera from cases of acute pneumonia showed nonspecific capsular swelling substance as a rule.

The eleven negative cases were ones of relatively mild bronchopneumonia. Only one blood sample was taken in their case. In the positive cases the titer for the substance varied between 1 and 32. Titers 4 and 8 each occurred in one third of the cases, titer 16 in a fifth and titer 32 in one case. No definite correspondence between the titers during the acute phase of the pneumonia and the severity of the infection was observed.

### Septicemia.

The cases of septicemia were thirteen in number. Five of them ran a violent course, leading to rapid death. The others, cases of subacute bacterial endocarditis, likewise ended in death eventually, although two of them were not followed until the end. Table 26 records the capsular swelling reaction as well as a number of clinical data.

The upper half of the table covers the acute cases. They showed a high content of nonspecific capsular swelling substance in the blood throughout the disease with the exception of case 120. In this case, where the symptoms were unusually severe with masses of bacteria in the blood during the last few days of life, the substance was lacking in a blood sample taken the day before death.

In the more chronic cases the content of nonspecific capsular swelling substance varied parallelly with the intensity of the symptoms. Case 122 is illustrated in figure 4.

*Case 122*, Mr. R. E. J—n, postman, aged 30, was treated for subacute bacterial endocarditis for a little over a year, during which time there was continuous bacteremia with alpha streptococci and repeated emboli to different organs. The figure gives a small part of the record to show variations in the capsular swelling substance during a period of active therapy. The treatment consisted of frequent blood transfusions and autovaccination previous to sulfapyridine therapy. The auto-vaccine contained about one milliard bacteria per milliliter and was given twice a week for a little over a month, thereafter weekly. A dose of 0.2 Ml. was given first, and the quantity gradually increased to 1 Ml. During this period the bacteria decreased in the blood

Table 26. — The occurrence of nonspecific capsular swelling substance in cases of septicemia.

Case No.	Diagnosis Acute cases	Etiology	Duration of disease	Highest values recorded			
				Evening temp. C.	S.R. in 1 hour	W.B.C.	N.C.S.
116	Quinsy + septicopyemia .	Beta Str.	6 days	40.4	19	—	16
117	Ulcerous endocarditis + acute meningitis . . . .	Staph. aureus	8 »	41.0	66	20,000	8
118	Ulcerous endocarditis . . .	Beta Str.	23 »	41.1	110	12,700	8
119	» » . . . .	P. type 2	16 »	40.8	125	18,900	8
120	Nephrolithiasis; pyonephrosis with abscess; perinephritis; perforating ulcer of the stomach . . . .	E. coli	18 »	40.3	51	24,000	0
121	Subacute bacterial endocarditis . . . . .	Alpha Str.	3 mos., 25 days	40.4	60	12,400	2
122	Do. do.	Alpha Str.	1 year, 4 mos.	See figure 4			8
123	Do. do.	Alpha Str.	3 mos.,	41.0	90	14,200	8
124	Do. do.	Alpha Str.	5 mos.	39.0	112	18,600	4
125	Do. do.	Beta Str. (weak hemo- lysis)	> 6 mos.	39.0	90	8,400	4
126	Do. do.	Alpha Str.	4 mos., 12 days	40.5	80	8,800	4
127	Do. do.	Alpha Str.	2½ mos.	39.7	92	8,400	4
128	Do. do.	Alpha Str.	> 3½ mos.	39.0	—	10,200	4

and on one occasion blood culture was negative. Later on the infection again progressed and the bacteremia increased. The capsular swelling substance decreased and became more sensitive to heat during the period of improvement and on one occasion it was absent. It is interesting that, despite the negative blood culture on October 4, activation of the process was indicated the next day by the reappearance of the capsular swelling and its insensitivity to heat. This was followed by an increase in the bacteria in the blood. The sedimentation rate remained practically constant the whole time. The sulfapyridine kept down the temperature, but it had no real therapeutic effect.

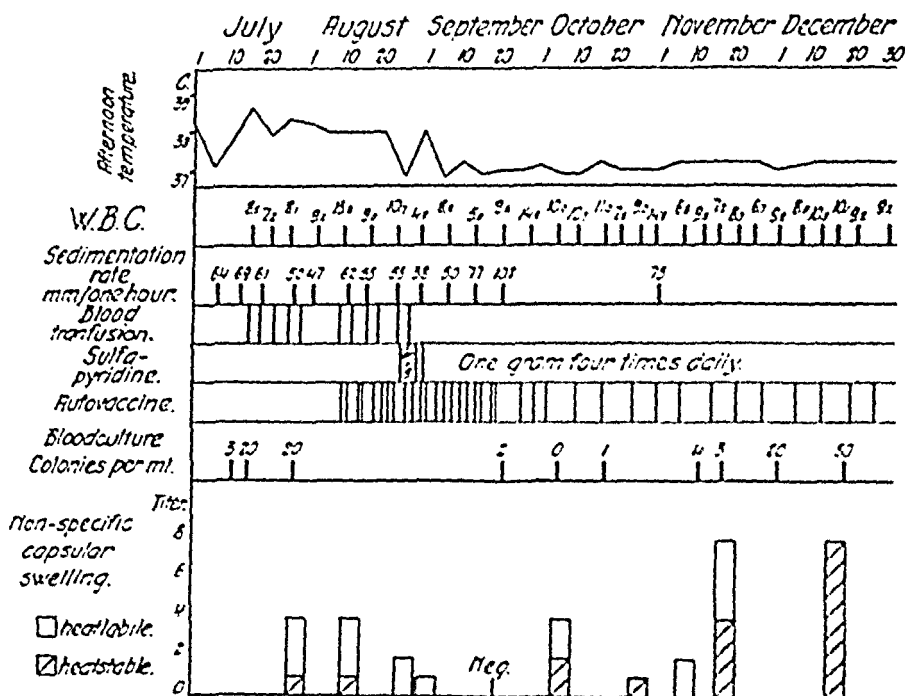


Fig. 4. Case 122, subacute bacterial endocarditis, man, 30 years of age. W. B. C. = white blood count in thousands. Heat-stable swelling substance is resistant to half an hour's heating at 65 C.

In the other cases the concentration of capsular swelling substance was low or it was lacking altogether during the quiescent stages of the infection, the content increasing as the infection grew more severe. In some cases the titer never exceeded 4, although tests were done during the last few weeks of life. In case 123 the high values were observed during periods of exacerbation.

### Salmonella Infections.

Salmonella infections are represented by nine cases of typhoid fever and two of paratyphoid fever. Table 27 shows these cases.

Eight cases with typhoid fever (129—136) showed bacterial growth in the blood; in one case (137) blood culture was negative. The agglutinin titer varied between 1/50 and 1/500, and nonspecific capsular swelling substance occurred in all except case 132, having a titer of 2 in two cases, of 4 in four cases and of 8 in two cases. There was no correspondence between the content of the substance and the specific agglutinin titer.



of spreading, causes either a mediastinitis anterior *via* lymphoglandulae sternales or a mediastinitis posterior (rare). This inflammation extends to the visceral pericardium aided by the gravitation,<sup>1</sup> the lift-and-force-pump action of the heart,<sup>2</sup> and the coughing and the pressing of the patient.<sup>3</sup> Thus the retrograde transport takes place. This peri-pericarditis irritates the visceral pericard and a pericarditis exsudativa results from an excessive secretion. In the same way a mediastinitis dextra or sinistra can originate by a sidelong descent of the inflammation with a successive pleuritis mediastinalis (Corning). A hematogenic origin of the pericarditis exsudativa is improbable. It cannot be assumed that the transport takes place along the tracheo-bronchial lymph vessels, the truncus bronchomediastinalis dextra or the ductus thoracicus to the angulus venosus and along the vena subclavia and the vena cava superior to the right heart, because there are no signs of myocarditis.

Assman<sup>4</sup> describes some cases of purulent mediastinitis superior, which were roentgenologically and autoptically confirmed, following an acute tracheo-bronchitis or after perforation of the tracheal wall by corpora aliena. In one case the formation of gas was observed in the mediastinum anterior as a result of contagion by putrid bacteria. Schinz<sup>5</sup>, too, gives some examples of such »Senkungsabscesse». v. Dehn<sup>6</sup> and Lorey<sup>7</sup> likewise observed some cases of mediastinitis anterior acuta and they point out the difficulties of a differential diagnosis with regard to tumors, thymus-hyperplasia etc. in the mediastinum anterior.

Nathan and Dathe put the question in their article referred to above, whether these clinically not very clear cases of pericarditis exsudativa might not be the cause of the local (or total) adhesive pericarditis which is sometimes found at autopsy (Smith and Willius<sup>8</sup>), whereas there was no evidence in the anamnesis either of the causative factor or of the pericarditis.

So it appears that the pericarditis and the attending mediastinitis anterior pass in many cases practically without any symptom and that only an accurate and systematic examination in

<sup>1</sup> David A. Nathan and Rich. A. Dathe, Amer. Heart J. 31, 115, 1946.

<sup>2</sup> Corning, H. K., Lehrbuch der Topografischen Anatomie. 1915.

<sup>3</sup> Braun, W., Topograf. anat. Atlas, 1924.

<sup>4</sup> Assman, H., Klin. Roentgendiagnostik, F. Vogel, 1934.

<sup>5</sup> Schinz, H., Dtsch. Z. Chir. 179, 1929.

<sup>6</sup> v. Dehn, W., Berl. Klin. Wschr. 480, 1910.

<sup>7</sup> Lorey, P., 8. Roentgenkongress.

<sup>8</sup> Smith, H. L., and Willius, F. A., Arch. Int. Med. 50, 410, 1932.

in only about a third of the cases. The disease or recurrence was reckoned to have begun at the time when distinct symptoms were observed, such as cough and fever, catching pain on respiration with fever, or only fever in the cases where tuberculous changes in the lungs were diagnosed later.

Table 28 gives a comparison between the symptoms and the nonspecific capsular swelling substance in the cases examined in the first and second week of illness.

Sera taken during the first week of the disease or of acute recurrence contained nonspecific capsular swelling substance in nine out of ten cases. The sera taken the second week of illness were more often negative, only three out of eight being positive, despite the fact that pulmonary changes, fever and the sedimentation rate showed that the disease had by no means entered an inactive stage.

Cases 184, 188 and 189 showed widespread lesions in the lungs but no capsular swelling substance in the blood. Case 190, rapidly leading to death, showed a large content of the substance.

To examine whether tuberculous processes with cavity formation offering large absorbing surfaces could exist without the substance occurring in the blood, thirty-two more chronic cases in which the sample was taken more than four weeks after the onset of the disease were collected. Eleven of them, with fever and widespread progressing changes in the lungs, are included in table 29. The other twenty-one, more inactive cases all lacked nonspecific capsular swelling substance.

Three cases in the table lacked the substance. In case 193 spontaneous pneumothorax occurred the day after the blood was taken and one week later the patient died. Autopsy showed a large ruptured cavity and widespread lesions in the lungs. Case 194 had bilateral pulmonary processes of great extent, a high fever and sedimentation rate, and a high white blood count. Extensive changes in the lungs also occurred in the other case, number 198, but the course was more drawn out.

If these three cases are compared with the other eight which showed the presence of nonspecific capsular swelling substance, it is seen that there was no essential difference between the two groups, either as regards the extent of the pulmonary lesions or as regards the clinical signs and symptoms.

Table 28. — Cases of pulmonary tuberculosis examined for nonspecific week of

Case No.	Cases examined during	
	Extent of pulmonary changes	
	Date	Clinical data, roentgen observations, etc.
172	8/3	X-ray: Left-sided pleurisy, old and new apical lesions.
173	29/3	X-ray: Large exudate in left pleura; parenchymal lesions of the right lung.
174	29/3	X-ray: Central parenchymal infiltration on the left side.
175	16/6	X-ray: Right-sided pleurisy and parenchymal infiltration infra-clavicularly on the same side.
177	26/7	X-ray: Right-sided pleurisy.
178	8/9	X-ray: Focus of spotty and linear consolidations the size of the palm of a hand in the right first and second I.S.
179	30/11	X-ray: Dense consolidation in the right upper lobe caused mainly by infiltration but also by atelectasis.
180	30/12	X-ray: Large pneumothorax laterally at the base of the right lung. Old fibrotic, partly calcified tuberculous foci in the upper half of the left lung. A number of small rarefied areas, indicating cavities, especially infraclavicularly.
181	16/6	X-ray: The right base filled up to the 2nd I.S. with a dense pleural effusion.
182	27/2	X-ray: Small consolidations in the left apex of tuberculous nature; widespread, recent consolidations in the lower lobe (nonspecific?).
Cases examined during		
183	4/3	X-ray: Parenchymal changes in the left lung, mainly in the upper lobe.
184	2/4	X-ray: Bilateral veil-like confluent parenchymal infiltration.
185	11/7	X-ray: Dense consolidation in the right lower lobe; parenchymal infiltration in the middle of the left lung.
186		Left-sided partial thoracoplasty; recurrence at the end of July.
187	24/10	X-ray: Left-sided pleural exudate increased (first x-ray October 20), covering nearly whole left chest.
188	21/12	X-ray: Right-sided basal pleural effusion up to 4th I.S. On the left side a bean-sized rarefaction, indicating cavity in the 1st I.S.; fairly dense parenchymal consolidation in the upper part. Progress.
189	26/12	X-ray: Bilateral, spotty consolidations with signs of several small cavities.
190	18/1	X-ray: Bilateral tuberculous changes with cavity formation in the right apex.

capsular swelling substance in the blood during the first and second illness.

the first week of illness								
Evening temp.		W.B.C.		S.R.		N.C.S.		Subsequent course
Date	C.	Date	No.	Date	mm./hr.	Date	Titer	
$\frac{8}{3}$	39.3	$\frac{8}{3}$	8,000	$\frac{14}{3}$	96	$\frac{8}{3}$	4	Death
$\frac{23}{3}$	38.6	$\frac{29}{3}$	8,600	$\frac{29}{3}$	85	$\frac{29}{3}$	8	Home
$\frac{8}{6}$	39.2	$\frac{6}{6}$	4,700	$\frac{8}{6}$	18	$\frac{8}{6}$	1	Sanatorium
$\frac{15}{6}$	38.7	$\frac{16}{6}$	6,200	$\frac{16}{6}$	48	$\frac{19}{6}$	c	Sanatorium
$\frac{23}{7}$	39.1	$\frac{26}{7}$	5,400	$\frac{27}{7}$	52	$\frac{29}{7}$	1	Sanatorium
$\frac{7}{9}$	39.2	$\frac{8}{9}$	10,200	$\frac{7}{9}$	65	$\frac{12}{9}$	1	Sanatorium
$\frac{23}{11}$	39.0	$\frac{23}{11}$	7,400	$\frac{27}{11}$	26	$\frac{23}{11}$	2	Sanatorium
$\frac{15}{12}$	39.3	$\frac{13}{12}$	6,000	$\frac{13}{12}$	43	$\frac{15}{12}$	4	?
$\frac{17}{6}$	39.4	$\frac{15}{6}$	4,900	$\frac{15}{6}$	56	$\frac{17}{6}$	4	?
$\frac{26}{2}$	39.2	$\frac{27}{6}$	6,400	$\frac{27}{2}$	37	$\frac{27}{2}$	0	?

the second week of illness

$\frac{3}{3}$	38.5	$\frac{4}{3}$	8,400	$\frac{4}{3}$	70	$\frac{4}{3}$	0	Sanatorium
$\frac{1}{4}$	39.6	$\frac{3}{4}$	2,600	$\frac{1}{4}$	8	$\frac{2}{4}$	0	Death
$\frac{17}{7}$	38.8	$\frac{16}{7}$	5,700	$\frac{11}{7}$	90	$\frac{15}{7}$	0	Sanatorium
$\frac{8}{8}$	38.8	$\frac{9}{8}$	16,800	$\frac{9}{8}$	52	$\frac{8}{8}$	2	Sanatorium
$\frac{27}{10}$	39.5	$\frac{27}{10}$	4,600	$\frac{30}{10}$	60	$\frac{27}{10}$	4	Sanatorium
$\frac{21}{12}$	39.0	$\frac{21}{12}$	2,500	$\frac{20}{12}$	40	$\frac{21}{12}$	0	At home
$\frac{25}{12}$	39.5	$\frac{23}{12}$	8,500	$\frac{27}{12}$	41	$\frac{29}{12}$	0	Sanatorium
$\frac{24}{1}$	39.2	$\frac{22}{1}$	10,200	$\frac{23}{1}$	140	$\frac{23}{1}$	8	Death

Table 29. — (Chronic cases of pulmonary tuberculosis examined for weeks after the onset

Case	Data from history	
	Date	Clinical data, roentgen observations, etc.
191	1939 20/11	X-ray: Right-sided pleural effusion and parenchymal lesions of uncertain nature.
192	1938 1939	November: Pneumothorax on left side. January: Poor condition, Cachectic. May: Spread to right lung. July: Progress on right side. November: Progress seen in roentgen picture.
193	1937 1939 11/10 12/10 10/11	Tuberculosis of right lung. X-ray: June 7: Progress on left side where large cavity was seen. Pneumothorax on left side. X-ray: Large cavity suspended in adhesions on left side. High temperature and signs of rupture of cavity.
194	1939	Treated for large bilateral lesions with cavities since April 1.
195	1936 1939	Incipient pulmonary tuberculosis. X-ray showed: Left lung collapsed; extended cloudy consolidations over the greater part of the right lung field.
	21/10 7/12	X-ray showed progress on the right side. Diffuse facial cyanosis on slightest exertion.
196	1939 12/1 10/11	X-ray: Hazy confluent consolidations with large cavities. Increasing debility; abdominal pain and diarrhea.
197	1935 1939 12/11	Beginning of pulmonary tuberculosis. X-ray: Diffuse consolidation in the greater part of the left lung with air-containing areas; cavities?
198	1927 1939 7/1 21/1 6/1	Beginning of pulmonary tuberculosis. X-ray: Two large cavities in the right apex. Pneumothorax on left side with exudate. X-ray: Progress on right side. Poor general condition.
199	1939 12/10	Pulmonary disease began in spring. X-ray: Extended bilateral lesions, mostly on the left side, with possible cavity formation in apices.
200	1933 1939 5/6 14/6 27/10 12/11	Beginning of pulmonary tuberculosis. Recurrence in June. X-ray showed left-sided consolidation. Pneumothorax on left side. X-ray showed exudate on right side. X-ray showed increase of exudate.
201	1935 1939 14/11	Beginning of pulmonary tuberculosis. Recurrence in October. X-ray: Extended bilateral lesions with large cavities.
17	17/11	Laryngeal tuberculosis.

nonspecific capsular swelling substance in the blood more than four of the disease.

Evening temp.		W.B.C.		S.R.		N.C.S.		Subsequent course
Date	C.	Date	No.	Date	mm./hr.	Date	Titer	
<sup>11</sup> / <sub>12</sub>	38.2	<sup>11</sup> / <sub>12</sub>	4,700	<sup>6</sup> / <sub>12</sub>	78	<sup>11</sup> / <sub>12</sub>	4	Sanatorium
<sup>15</sup> / <sub>11</sub>	38.5	<sup>15</sup> / <sub>11</sub>	13,200	<sup>15</sup> / <sub>11</sub>	36	<sup>15</sup> / <sub>11</sub>	2	Died <sup>16</sup> / <sub>1</sub> 1940
<sup>9</sup> / <sub>11</sub>	38.2	<sup>9</sup> / <sub>11</sub>	13,400	<sup>9</sup> / <sub>11</sub>	25	<sup>9</sup> / <sub>11</sub>	0	Died <sup>14</sup> / <sub>11</sub> 1939
<sup>22</sup> / <sub>11</sub>	39.4	<sup>22</sup> / <sub>11</sub>	13,600	<sup>22</sup> / <sub>11</sub>	104	<sup>22</sup> / <sub>11</sub>	0	Died <sup>12</sup> / <sub>2</sub> 1940
<sup>1</sup> / <sub>11</sub>	39.3	<sup>1</sup> / <sub>11</sub>	9,600	<sup>1</sup> / <sub>11</sub>	61	<sup>1</sup> / <sub>11</sub>	8	Died <sup>27</sup> / <sub>12</sub> 1939
<sup>1</sup> / <sub>11</sub>	39.5	<sup>1</sup> / <sub>11</sub>	15,000	<sup>1</sup> / <sub>11</sub>	20	<sup>1</sup> / <sub>11</sub>	8	Died <sup>4</sup> / <sub>12</sub> 1939
<sup>16</sup> / <sub>11</sub>	38.2	<sup>16</sup> / <sub>11</sub>	7,200	<sup>16</sup> / <sub>11</sub>	21	<sup>16</sup> / <sub>11</sub>	4	Continued hospital care
<sup>30</sup> / <sub>11</sub>	38.0	<sup>30</sup> / <sub>11</sub>	9,800	<sup>30</sup> / <sub>11</sub>	51	<sup>30</sup> / <sub>11</sub>	0	Died <sup>30</sup> / <sub>9</sub> 1940
<sup>16</sup> / <sub>11</sub>	37.7	<sup>16</sup> / <sub>11</sub>	10,600	<sup>16</sup> / <sub>11</sub>	53	<sup>16</sup> / <sub>11</sub>	2	Continued hospital care
<sup>11</sup> / <sub>11</sub>	39.9	<sup>11</sup> / <sub>11</sub>	7,800	<sup>11</sup> / <sub>11</sub>	88	<sup>11</sup> / <sub>11</sub>	4	Continued hospital care
<sup>22</sup> / <sub>11</sub>	37.8	<sup>22</sup> / <sub>11</sub>	18,000	<sup>22</sup> / <sub>11</sub>	56	<sup>22</sup> / <sub>11</sub>	2	Died <sup>26</sup> / <sub>12</sub> 1939

## Meningitis.

The cases of bacterial meningitis were severe and three of them, caused by *Pneumococcus* type 8 (140) and 18 (141), and "intracellular cocci" (142), respectively, ended in death. In another three, one of meningococcic meningitis (143), one of meningitis caused by "gram-positive cocci" (144) and one caused by pneumococci of type 22 (145), the patients recovered. In all the cases the nonspecific capsular swelling reaction was strongly positive, the titer varying between 8 and 32. The highest titer, 32, was found in a boy of six months, who died of type 18 pneumococcic meningitis (141).

The cases of abacterial meningitis are composed of one case of purulent meningitis (146) with no bacteria in the cerebrospinal fluid, two cases of cerebrospinal syphilis (147 and 148) and three of encephal meningitis of indefinite character (149, 150, 151). Nonspecific capsular swelling substance was lacking in all these cases.

Two cases of tuberculous meningitis were examined. In one case (152) the disease ran a rapid, malignant course and the patient died on the fourteenth day. Autopsy showed tuberculous meningitis in the tract of the hypothalamus. Nonspecific capsular substance was not observed in the serum from this patient. In the second case (153) the disease lasted about a month. The patient was admitted to the hospital five days before death, and lumbar puncture showed an initial pressure of 350 mm., a large amount of protein in the cerebrospinal fluid and 40 cells per cubic millimeter. Nonspecific capsular swelling substance was lacking both in the blood and cerebrospinal fluid. A sample taken the same day the patient died revealed a nonspecific substance titer of 2. Autopsy confirmed the diagnosis of tuberculous meningitis.

## Virus Diseases.

Seventeen of the 18 cases of poliomyelitis (154—171) were lacking in nonspecific capsular swelling substance. In the eighteenth case (154), one of fatal respiratory paralysis, only a faintly positive reaction of the (c) type was observed. In this case the serum was taken the same day the respiratory paralysis set in, difficult breathing and rales developing as the day went on. Coughing had set in earlier. Another of the cases (155) also ended in death from respiratory paralysis, but in this instance no capsular swelling was observed. The blood was taken on the fourth

day of illness, on the same day the breathing difficulties increased, and two days later death ensued. In the other cases, all of them with paralysis, the blood samples were taken during the febrile phase, during the first week in eight cases and after the disease had begun to subside in seven cases.

Blood was taken from twenty-two military recruits revaccinated against smallpox at the time the vesicle had reached the height of its development (table 25). Only one case showed nonspecific capsular swelling substance.

### Cardiac and Vascular Diseases.

In the cases of non-infectious cardiac and vascular diseases the acute cardiac infarction was always accompanied by nonspecific capsular swelling substance in the blood (table 25). Figure 5 illustrates a typical case (case 202).

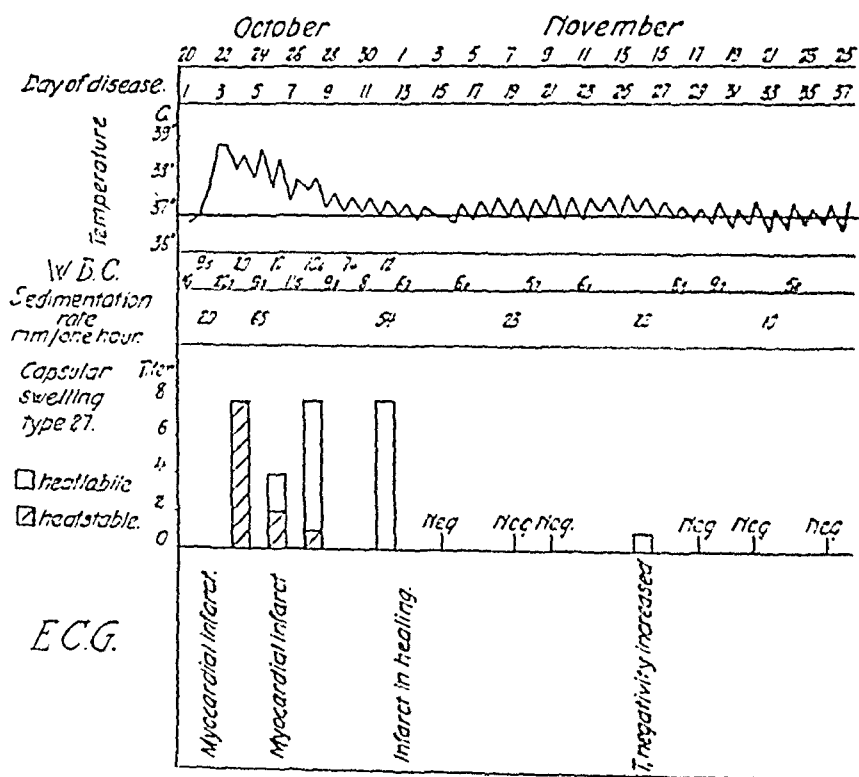


Fig. 5. Case 202, myocardial infarction, man, 62 years of age. W. B. C. = white blood count in thousands. Heat-stable swelling substance is resistant to half an hour's heating at 65 C. E. C. G. = electrocardiogram.



*Case 202, Mr. G. M. E. I—k, businessman, aged 62.*

The disease ran a benign course and the symptoms disappeared rapidly. As appears from the diagram, the swelling was marked in the acute stage of the disease, which was also accompanied by a rise in the white blood count, the sedimentation rate and the temperature. On the fifteenth day of the illness the acute symptoms had subsided, and the swelling disappeared, although the sedimentation rate remained elevated. On the twenty-sixth day there was again a slight swelling reaction and at the same time the electrocardiogram showed an aggravation and the white blood count became elevated; there was no change in the clinical condition, however. The sedimentation rate changed only slightly. Possibly these signs were caused by an extension of the infarction too slight to cause subjective symptoms.

In the other cases of cardiac infarction (91, 203—207), the content of nonspecific capsular swelling substance varied parallelly to the other signs of the disease.

Circulatory disorders due to valvular defect or cardio-arteriosclerosis are not accompanied by nonspecific capsular swelling substance in the blood. Consequently the capsular swelling reaction may help to differentiate between cardiac infarction and cardiac decompensation without tissue disintegration.

Nonspecific capsular swelling substance was not observed in any of seven cases of nephrosclerosis with hypertension and albuminuria (table 25).

In two out of three cases of deep thrombosis examined, the process was seated in the lower extremities and in the third case in the arm. No capsular swelling substance was observed in any of these cases.

Two cases of pulmonary embolism and one of cerebral embolism were examined, all three with negative results.

One case of pulmonary embolism (86), however, has several interesting features and is worth a closer analysis.

*Case 86, Mr. R. B—l, language teacher, aged 63, was taken ill suddenly on Sept. 24, 1941, with chill, fever and cough. He continued working for ten days, but felt tired, perspired profusely and his body ached all over. He was admitted to St. Erik's Hospital on October 3. Roentgenography showed basal pneumonia on the left side, and the sputum contained pneumococci of type 3. He was given full doses of sulfathiazole from October 3 to 7. The temperature was 39 C. the first day, but then dropped lytically, the patient being subfebrile from October 8 on. The sedimentation rate remained over 100 mm. in one hour in repeated tests. The white blood count was 15,800 on October 3, 6,200 on October 8 and 12,700 on October 11. On October 23 the patient suddenly died. Autopsy revealed catarrhal pneumonia*

and bilateral pulmonary emboli, death being probably caused by the latter. The titer of the nonspecific capsular swelling substance was 8 on October 4 and 4 on October 7, 8 and 11. Blood taken after death contained specific antibodies against type 3 pneumococci, but no capsular swelling substance.

*Summary.* A patient convalescing from type 3 pneumonia died from pulmonary embolism. Specific agglutinins were formed against type 3 pneumococci. Nonspecific capsular swelling substance appeared during the acute stage of the pneumonia, but disappeared from the blood when recovery took place, being unaffected by the formation of thrombi which probably preceded the embolism.

### Cases of Tumor.

A few cases of different kinds of tumor were examined, including eight cases of mammary cancer. The difficulty with these cases is that, when nonspecific capsular swelling is observed, it is impossible to exclude the possibility of its being induced by secondary bacterial infection in the disintegrating tumorous tissue. Some of these cases will now be commented upon.

*Case 208,* Mr. K. F. N—n, printer, aged 55, began to feel tired and to cough in the beginning of 1939. He fell ill suddenly on June 8, 1939, with severe stitch in the left side of his chest, and was admitted to St. Erik's Hospital on June 12. Roentgenography showed moderately enlarged hilar shadows and increased markings at the bases of the lungs. Re-examination on June 28 and July 7 showed extension of the changes and on July 31 a basal consolidation covering the whole pulmonary field up to the third intercostal space was observed. Bronchography on August 15 showed narrowing of the main bronchus of the right lower lobe, and bronchoscopy on August 25 revealed a slightly bleeding tumor at this site. It proved to be a squamous cell carcinoma on histologic examination. The temperature was subfebrile during the hospital stay, except for short periods when it lay between 38 and 39 C. The sedimentation rate was 95 mm. in one hour on June 10, 120 mm. on July 17 and 142 mm. on August 17. The white blood count varied between 7,200 and 14,700. The serum showed a titer of 1 for nonspecific capsular swelling substance on July 17.

*Summary.* A case of bronchial carcinoma with atelectasis, pleural effusion and a high sedimentation rate showed a very weak nonspecific capsular swelling reaction.

One case of hypernephroma (209) was followed with several blood tests during the course of the disease.

*Case 209,* Mr. A. E. J—n, machinist, aged 52, who had suffered since 1912 from an organic heart lesion, fell ill in March of 1941

with a common cold with fever. The temperature, which lay between 37 and 38.5 C., did not return to normal when the other symptoms disappeared, and the patient felt tired and lost weight. He was admitted to St. Erik's Hospital on April 25, 1941. The temperature was then 38.4 C., the white blood count 9,900, the red blood cells, 3,840,000, the hemoglobin 64 per cent and the sedimentation rate 118 mm. in one hour. The urine contained no pathologic components and blood cultures were negative. The patient remained in about the same condition for the next few months. He continued to lose weight and felt tired. Pyelography was done repeatedly but signs of a renal tumor were first observed on September 16. On left-sided nephrectomy on September 24 a large hypernephroma was removed. The patient recovered rapidly and was discharged from the hospital on November 12. Nonspecific capsular swelling substance was present on June 7 and 11, having a titer of 8, but absent on October 7 and 27.

*Summary.* A man of 52 was operated upon for a hypernephroma after six months of fever, high sedimentation rate, secondary anemia, fatigue and loss of weight. The operation was followed by rapid recovery. Before the operation nonspecific capsular swelling substance was present with a titer of 8 but afterwards it disappeared.

Three other cases of advanced malignant tumor were examined.

*Case 210,* Miss E. I. T—p, aged 44, had been treated for gastritis for ten years. In the middle of May 1942 her discomfort increased and she lost appetite and weight. She was admitted to St. Erik's Hospital on June 22, pale, tired and showing dried skin and fissures at the corners of her mouth. Ascites was found. The red and white blood corpuscles and hemoglobin showed normal values, and the sedimentation rate was 16 mm. in one hour. The temperature was normal on admission but was occasionally subfebrile afterwards. Roentgenography pointed to carcinoma in the canalis part of the stomach. Laparotomy confirmed the diagnosis. The peritoneum was lined with metastatic nodes and the omentum was converted into a firm plaque adhering to the anterior abdominal wall. The nonspecific capsular swelling reaction was negative on July 5.

*Summary.* A case of gastric carcinoma with extensive metastases to the peritoneum and ascites, showed no nonspecific capsular swelling reaction.

*Case 211,* Mr. K. T. E—m, transport worker, aged 59, was admitted to St. Erik's Hospital on March 12 for left-sided pneumonia and a tendency to nose-bleeding. The blood appeared normal on entry, but after recovery from the pneumonia anemia without any regenerative signs developed. Sternal puncture on May 19 revealed cancer metastases and the primary tumor was traced to the prostate on May 23. Roentgenography showed metastasis to the bones of the pelvis, the femur and the lumbar spine. Roentgen treatment was given because of severe pain on May 28 and 29 and June 5, 6 and 7 and every second day thereafter. On June 11 the nonspecific capsular

swelling reaction was negative, the temperature normal, the white blood count normal and the sedimentation rate 34 mm. in one hour. The red blood cells numbered 3,380,000 and the hemoglobin was 59 per cent.

*Summary.* In one case of prostatic carcinoma with generalized metastases to the bones and aplastic anemia, the nonspecific capsular swelling reaction was not observed despite roentgen treatment for two weeks.

*Case 212.* Mr. R. H. T—e, caretaker, aged 26, felt tired in the spring of 1941. In June he began to suffer from pain in his feet, shoulders, elbows and wrists, enlargement of the glands on his neck, diminished hearing in his left ear, reduced sensitivity in the right corner of his mouth, difficulty in swallowing and a feeling of fever. He was admitted to St. Erik's Hospital on June 14. Examination revealed pale skin with a tinge of grey, enlarged submandibular, axillary and inguinal lymph nodes, normal temperature and a sedimentation rate of 139 mm. in one hour. The white blood cells numbered 2,800, the red cells 2,830,000, and the hemoglobin measured 55 per cent. Examination of an excised lymph node gave the diagnosis — reticular cell sarcoma. Roentgen treatment was given on June 28 and 30 and July 5 and at intervals afterwards until the patient's death on Jan. 19, 1942. Autopsy confirmed the diagnosis.

On July 3 the nonspecific capsular swelling reaction was negative, the white blood count 2,400, the red blood count 3,590,000, the hemoglobin 85 per cent, the sedimentation rate 134 mm. and the temperature normal. On July 10 the blood plasma contained 10.9 per cent total protein, the globulin amounting to 6.8 and the albumin to 4.1 per cent.

*Summary.* A case of reticular cell sarcoma at an advanced stage with anemia and leukopenia, enlargement of the lymph nodes, trophic disorders, exceedingly high sedimentation rate and an elevated globulin content in the blood showed no nonspecific capsular swelling substance.

Eight cases of cancer of the breast (213—220), all treated at Radiumhemmet, were examined for nonspecific capsular swelling substance. Four of the tumors (213—216) had not metastasized and in two cases (217 and 218) operable axillary metastases were present. In another case (219) it was doubtful whether operation could be done because of a metastasis a little larger than a goose egg in the axilla, and the eighth case (220) was inoperable, with cancer in the left breast and metastases in both axillae and in the left supraclavicular fossa. None of these cases showed nonspecific capsular swelling substance.

## CHAPTER 7.

# The Relationship between Nonspecific Capsular Swelling Substance and Some of the Most Common Clinical Criteria.

## The Severity of the Disease.

In an earlier paper I pointed out that if nonspecific capsular swelling was demonstrable in acute pneumonia beyond the sixth to the twelfth day of the disease, there was reason to suspect complications. Table 30 covers 87 cases of acute pneumonia and compares the number of days of fever with the number of days in which the reaction was observed in the blood. The relation between the duration of the swelling and the frequency of complications is seen therefrom.

Table 30. — The relation between the number of days the capsular swelling persisted and the number of fever days as well as the development of complications in 87 cases of acute pneumonia.

No. of days N.C.S. present	No. of cases	Fever days (average)	Complications				Total
			Em-pyema	Clear exudate	Acute otitis	Serum disease	
1—5	7	6.6	0	0	0	0	0
6—10	34	9.6	0	1	1	1	3
11—15	28	14.0	0	3	1	0	4
16—20	3	15.0	0	0	0	0	1 <sup>1</sup>
> 20	15	32.2	3	6	0	1	10

<sup>1</sup> Pulmonary tuberculosis.

The number of fever days corresponds approximately with the days of positive capsular swelling. Complications appeared in cases with prolonged fever and a prolonged positive reaction. Fourteen cases of acute pneumonia ended fatally, and the swelling was demonstrated immediately before death in all of them.

## The Presence of Specific Agglutinins.

A series of thirty-six cases (222—257) of acute pneumonia treated with sulfapyridine were studied in order to ascertain the relationship between the presence of nonspecific capsular swelling substance and specific agglutinins in the blood. Table 31 covers these cases.

Table 31. — Relation between the nonspecific capsular swelling substance and specific agglutinins in 36 cases of acute pneumonia. All the figures refer to the number of days after the onset of disease.

Case	Etiology	First afeb- rile day	Tempor- arily afebrile	N.C.S.			Specific aggl'n's	Comments
				1st posi- tive test	Tempor- arily neg.	Change from pos. to neg.	Change from neg. to pos.	
222	P. type 1	8	3-4	3	—	6-9	6-9	
223		6	—	5	—	7-9	5-7	
224		4	—	3	—	5-12	5-12	
225		11	—	6	—	8-14	6-8	
226		9	—	7	—	9-13	9-13	
227		5	—	4	—	9-12	4-9	
228		14	—	9	6	9-11	6-9	
229		4	—	4	—	6-8	6-8	
230	P. type 3	9	—	5	—	11-13	8-11	
231		13	5 & 11	4	—	6-10	6-10	
232		10	—	5	—	9-14	5-7	
233	P. type 4	9	—	7	—	7-9	9-11	
234		7	—	6	—	9-11	9-11	
235		6	—	4	—	4-8	4-8	
236		9	—	4	—	4-8	4-8	
237		11	—	4	—	4-6	11-12	
238		22	—	4		7-14	7-14	Exudate puncture 20th day
239	P. type 5	8	—	2	—	5-11	5-11	
240		4	—	4	—	4-7	2-4	
241		35	15-21	5	20-27	31-33	3-5	See fig. 6
242		41	11-15 & 31	5	8	12-14	8-12	Empyema
243		4	—	7	—	7-10	7-10	
244		20	—	9	—	13-15	9-13	
245	P. type 7	7	—	4	—	5-6	6-8	
246		6	—	6	—	7-9	7-9	
247		7	—	6	—	7-11	7-11	
248	P. type 8	4	—	2	—	3-6	3-6	
249		7	—	3	1	6-9	3-6	
250		13	5	3	—	9-11	6-9	Joint pains 6-8th day
251	P. type 9	5	—	2	—	10-12	5-8	
252		15	7-11	4	—	4-7	4-7	
253	P. type 10	6	—	4	—	4-8	8-15	
254	P. type 12	7	4	3	—	3-8	12-15	
255		17	11	6	—	6-12	6-12	
256	P. type 18	11	6-8	3	—	4-6	6-9	
257	P. type 22	14	6-13	4	—	6-8	6-8	

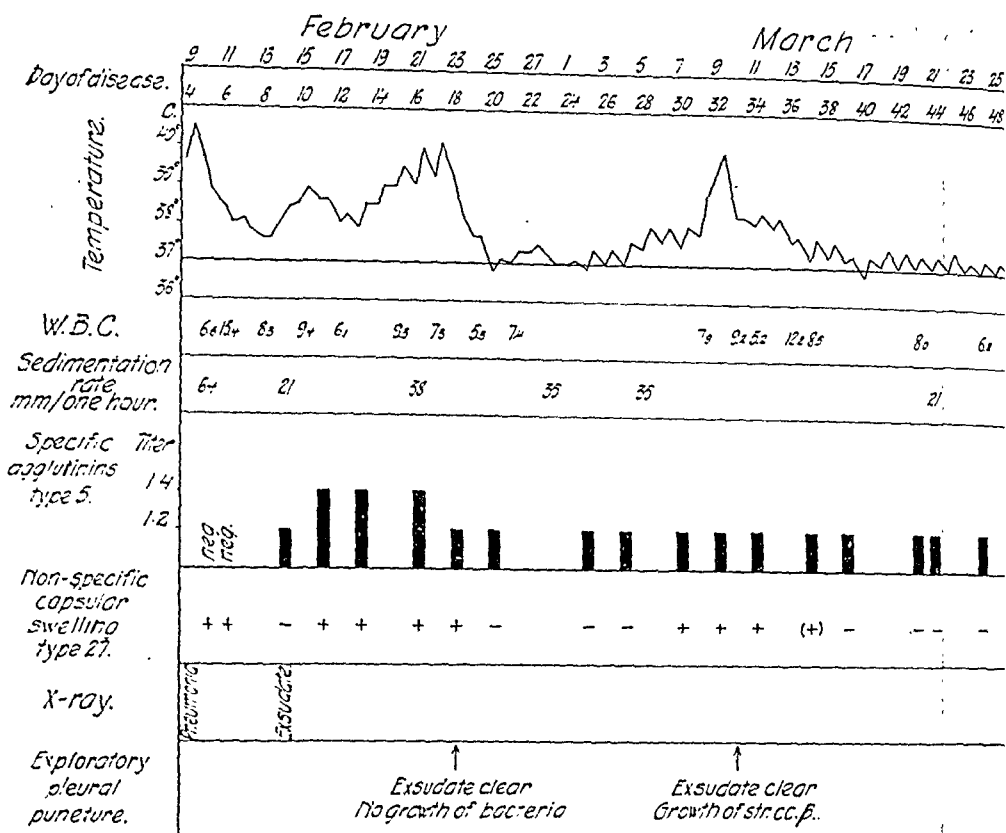


Fig. 6. Case 241, acute pneumonia caused by *Pneumococcus* type 5 and complicated by an empyema caused by beta hemolytic streptococci, man, 43 years of age. W. B. C. = white blood count in thousands.

In seventeen cases the capsular swelling substance disappeared from the blood at about the same time as specific agglutinins appeared. In six cases it disappeared a day or so before and in thirteen cases a day or so after the appearance of the agglutinins. In the last-mentioned cases, accordingly, agglutinins and non-specific capsular swelling substance were present simultaneously in the serum.

Figure 6 illustrates case 241, one of *Pneumococcus* type 5 pneumonia, complicated with streptococcic empyema.

Case 241, Mr. H. V. S.—n, electrician, aged 43, fell ill suddenly on February 6, 1939, with chill, fever and stitch in the chest. He was sent to St. Erik's Hospital on February 9 where roentgen examination revealed left-sided pneumonia. The subsequent course is seen from the figure.

Summary. A man of 43 fell ill from type 5 pneumonia, which was accompanied by nonspecific capsular swelling substance in the blood.

The substance disappeared when type 5 agglutinins appeared but returned during two later periods when the course was complicated by streptococcic empyema. It is interesting to note how the substance disappeared immediately after the removal of the exudate. After the first puncture the blood remained free from it for some time. When the exudate reappeared the substance returned. After the second puncture the process healed and the substance did not reappear.

In eight cases of salmonella infections (table 27), capsular swelling substance, bacteria and specific agglutinins were simultaneously present in the serum.

### Bodily Temperature.

It was earlier pointed out that the content of nonspecific capsular swelling substance fluctuates largely parallelly to the bodily temperature in acute cases, but in my previous study I also demonstrated that the capsular swelling in infections of the upper respiratory tract was not necessarily related to bodily temperature. My later investigations confirm this observation. Figure 4 shows that the substance may appear during periods of practically normal temperature.

Table 32.—The relation between capsular swelling and sedimentation rate and white blood count in acute pneumonia and pulmonary tuberculosis.

Sedimentation rate in one hour	Acute pneumonia							Pulmonary tuberculosis						
	Positive						Neg.	Positive						Neg.
	16	8	4	2	1	Not titr.		8	4	2	1	Not titr.	Total	
2—4					1		1	2					0	1
4—8							0	3					0	3
8—16			1	1		3	5	15					0	5
16—32					1	7	8	24		2			3	9
32—64	1			1	2	16	20	33	1	1	3	1	7	12
64—128		3	2	1		21	27	12	1	2	2		6	27
White Blood Count														
3—7 Thousand		1	3	2	3	28	37	29		2	1		3	15
8—12 "	1	4	5	3	2	37	52	62	2	2	2		4	16
13—17 "		2	6		3	26	37	20		2	1		5	6
18—22 "	1	1	3	1	1	23	30	9		1	1	1	3	0
> 22 "	1	2	2		1	26	32	5					2	0



## **The Sedimentation Rate and White Blood Count.**

Table 32 shows the relation of nonspecific capsular swelling to different sedimentation rates and white blood counts in cases of acute pneumonia and pulmonary tuberculosis.

The reaction did not vary precisely with the sedimentation rate or white blood count. However, it showed a general tendency to increase in frequency when elevated values for the latter were present.

## **CHAPTER 8.**

### **The Occurrence of Nonspecific Capsular Swelling Substance in Other Bodily Fluids than Serum.**

#### **Pleural Exudate.**

Pleural exudate was examined for nonspecific capsular swelling substance in a number of cases and the results are seen in table 33. The days the specimens were taken are given in relation to the day the illness set in.

Cases 258 to 268 had abacterial exudates. The exudate contained nonspecific capsular swelling substance in eight of these cases. In two of them, 258 and 260, the titer of the substance was not estimated, and in two others, 266 and 268, the titers in the serum and exudate were the same. In the four remaining cases, 259, 261, 264 and 265, there was a higher content of the substance in the serum than in the exudate. In cases 262, 263 and 267 the substance was present in the blood but not in the exudate. The rapid fluctuations of the substance in the blood are seen from case 265 where, after the pleuritic exudate had been removed, a titer of 8 disappeared in one day.

In cases 269 to 277 and case 241, bacteria were cultivated from the exudate. Nonspecific capsular swelling substance was lacking in the exudate in all the cases except 241 and 272, despite a high content in many of the blood samples. The reaction changed from positive to negative in cases 270 and 276 at the same time as it became possible to cultivate bacteria from the exudate. Case 276 illustrates well how the substance increased in the serum

Untersuchungen mit dem Tillmann'schen Verfahren herausstellten, der Ab- bzw. der Zunahme des Ascorbinsäuregehaltes im untersuchten Material zuzuschreiben sind.

Bei Beurteilung des Nikotinsäurehaushaltes musste berücksichtigt werden, dass von 100 mg nach Bandier's Vorschrift peroral eingeführter Nikotinsäure in 24 Stunden insgesamt 14 % ausgeschieden wird, wovon 70 % in den ersten 3 Stunden erscheint. Hingegen berichten Ellinger u. Shattock in ihrer bereits erwähnten Arbeit nach Belastung von 100 mg nur über eine Zunahme der Nikotinsäureausscheidung um 4—5 mg. binnen 24 Stunden, also in noch geringerem Verhältnis als in den Untersuchungen Bandiers. Da jedoch bei Untersuchung kleiner Mengen grosse Fehlermöglichkeiten nicht zu vermeiden sind, hatten wir parallel mit den Belastungsproben auch die Bestimmung der Nikotinsäure im 24-stundenharn ohne Belastung ausgeführt. Die Angaben im Schrifttum weichen auch hierüber ziemlich auseinander. So fanden Pearson u. Winegar, Harris u. Raymond, wie auch Swaminathan Tagesdosen von 5—6, Bandier solche von 2—3 mg. Alle Autoren wie auch Ellinger und Shattock stimmen darin überein, dass bei Nikotinsäuredefizienz die Ausscheidung täglich um 1 mg und noch darunter gefunden wird. Bei so widersprechenden Angaben des Schrifttums kann somit von einem normalen Nikotinsäuregehalte des Urins nicht gesprochen werden. Wir wandten zur Bestimmung die Anilin-Bromcyan-Methode von Pearson und Winegar mit dem Stufapparat von Pülfrich an; da jedoch Bandier nachwies dass von den Nikotinsäurederivaten Nikotinsäureamid, Cozymase, Coenzym und besonders die Nikotinursäure nur nach Hydrolyse mit starker Lauge für die Bestimmung erfassbar werden, haben wir Pearson und Winegar's Verfahren hiermit ergänzt.

Bei Vergleich der Ergebnisse nach Belastung mit Vitamin B<sub>1</sub> stellt sich heraus, dass die an unserer Klinik übliche Diät allein nach der Methode von Magyar keine B<sub>1</sub>-Hypovitaminose verursacht, ebensowenig entsteht Mangel nach längerer Behandlung mit Sulfonamiden. Bei Penicillinkuren haben wir die Belastung nach Einverleibung von 5—5.5 Millionen O. E. ausgeführt, nur ein Patient erhielt bei der Untersuchung 11 Millionen Einheiten. Wie wir anlässlich des Vitamins B<sub>2</sub> bereits erwähnten, ist infolge des verminderten Wertes der derzeitigen Ernährungsverhältnisse ein geringer Mangel an B<sub>1</sub> auch bei gesunden Menschen zu finden, so müssen die am Anfang der Penicillindosierung gefundenen Aus-

Table 33. — The content of nonspecific capsular

Case No.	Etiology	Pleural			
		Day of test	Appearance	Cells per mm <sup>3</sup>	Protein cont.
258	P. type 1	13	Empyema	8200	—
259	P. type 1	19	Opalescent	11600	4.4 %
260	P. type 3	16	Opalescent	10000	—
261	P. type 3	35	Opalescent	4800	1.5 %
262	P. type 16	6	Opalescent	—	—
263	B. Friedländer	31	Bloody	—	—
	+ T.b.	36	Clear	1600	—
264	T.b.	11	Bloody	—	—
265	T.b.	35	Clear	200	2.5 %
		—	—	—	—
266	Unknown	11	Clear	1200	1.5 %
267	Unknown	240	Clear	200	1.2 %
268	Unknown	7	Clear	—	—
269	P. Type 1	7	Bloody, cloudy	—	—
241	P. type 5	36	Clear	3000	—
270	P. type 9	8	Opalescent	5100	—
		19	Opalescent	200	—
		41	Empyema	—	—
271 <sup>1</sup>	P. type 9	59	Empyema	—	—
		60	Empyema	—	—
		61	Empyema	—	—
272	T.b.	5	Clear	3200	2.0 %
		23	Clear	5400	—
273	Alpha Str.	10	Clear	1	1
274	Staphylococci	21	Empyema	—	—
275	Anaerobic rods	8	Empyema	—	—
276	Gram-labile cocci+	16	Clear	1	1
	gram-negative rods	23	Empyema	1	1
277	P. type 5	38	Empyema	—	—
177 <sup>1</sup>	T.b.	5	Clear	6000	—
		17	Clear	—	—

<sup>1</sup> Given antipneumococcic serum type 9 intravenously.

On August 22 another intrapleural injection of 40,000 I. U. was given, and it was followed by capsular swelling of the pneumococci and on August 23 complete phagocytosis was observed. On August 26 the pleural pus was again free from mobile antibodies and contained a moderate amount of freely mobile pneumococci.

Pneumococci could be cultivated from the pus, even from the specimens which showed capsular swelling and phagocytosis.

swelling substance in pleural exudate.

exudate			Serum		
Bacterial growth	N.C.S. titer	Spec. aggl. titer <sup>1</sup>	Day of test	N.C.S. titer	Spec. aggl. titer <sup>1</sup>
0	c+++	0	16	c+++	0
0	2	—	19	8	—
0	(c)+++	0	15	c++++	0
0	4	—	35	8	—
0	0	0	6	2	0
0	0	—	31	c+	—
0	0	—	37	c+	—
0	1	—	11	2	—
0	4	—	35	8	—
—	—	—	36	0	—
0	4	—	11	4	—
0	0	—	240	4	—
0	8	—	7	8	—
Type 1	0	0	7	c++++	0
Beta Str.	(c)	1/2	37	c	1/2
0	(c)	0	7	c+	0
0	c	1/16	18	c+	1/16
Type 9	0	1/8	39	c+	1/8
Type 9	0	0	59	16	1/16
Type 9	0	0	60	1	1/8
Type 9	0	0	61	4	1/16
T.b.	2	—	5	4	—
0	0	—	33	0	—
Alpha Str.	0	—	10	8	—
Staph.	0	—	21	16	—
Anaerobic rods	0	—	8	8	—
0	4	—	16	8	—
Putrid growth	0	—	23	16	—
Type 5	0	0	32	0	1/16
			50	0	1/8
T.b.	0	0	6	1	0
—	0	1/32	17	0	1/32

<sup>1</sup> Tested with bacterial density 16.

The patient's temperature was subfebrile for the most part from July 14 to August 8, but showed occasional peaks to 40 C. From August 9 to 14 the temperature varied between 37 and 39 C. and from August 15 on, it was normal. The sedimentation rate was 82 mm. on July 19, 99 mm. on August 2, 82 mm. on August 16 and 50 mm. on August 23. On September 19 the patient was discharged well. Resection of a rib had been considered, but did not need to be done.

*Summary.* A man of 38 acquired type 9 empyema, after recovering from an operation for pancreatitis. He was given serum treatment first intravenously and then intrapleurally. The intravenous injections caused circulating antibodies in the blood, but did not exert any influence whatever on the empyematous fluid, where the agglutinin titer was negative and the bacterial growth apparently continued undisturbed. The intrapleural injection of serum caused immediate capsular swelling and agglutination of the pneumococci, followed a day later by maximal phagocytosis. A few days later the administered serum was neutralized and the infection progressed, with pneumococci swimming about freely among the white blood cells in the empyema. On renewed injection the same series of events occurred. Despite the brief effect of the intrapleural serum, the patient improved rapidly, perhaps also because of the many pleural punctures.

There does not appear to be any relationship between the presence of nonspecific capsular swelling substance in a pleural exudate and its cellular or protein content.

On dialysis of exudate containing the substance, the latter went over into the water-insoluble fraction.

### Cerebrospinal Fluid.

It was not possible to demonstrate nonspecific capsular swelling substance in the cerebrospinal fluid in bacterial (6 cases) or abacterial meningitis (4 cases) or in poliomyelitis (12 cases).

### Human Milk.

One case of type 1 pneumonia during pregnancy and childbirth enabled a study of both nonspecific capsular swelling substance and specific antibodies in human milk.

*Case 229,* Mrs. A. M. J—n, aged 28, quadripara, fell ill suddenly on May 3, 1940, in the ninth month of pregnancy with violent headache, vomiting and a temperature of 40.1 C. She was admitted to St. Erik's Hospital on May 4 with a temperature of 39.4 C., a pulse rate of 120, a blood pressure of 125 systolic and 75 diastolic, a white blood count of 29,800 and a sedimentation rate of 45 mm. in one hour. Roentgen examination revealed bronchopneumonic infiltration in the right lower lobe. The sputum contained pneumococci of type 1. The patient was given full doses of sulfapyridine. Delivery took place on May 4 at 10.50 p. m. and the patient then improved rapidly.

Nonspecific capsular swelling substance was present in the blood

with a titer of 8 on May 6 and of 4 on May 8 and was lacking from May 10 on. A specimen of milk taken on May 7 showed no sign of the substance.

Specific antibodies against type 1 appeared in the blood and milk on May 10 with a titer of  $1/4$ . They had the same titer in the blood on May 14 and a titer of  $1/2$  on May 17 and 22, but were lacking in the milk from May 14 on.

*Summary.* A woman of 28 fell ill a few days before delivery with type 1 pneumonia, and recovered rapidly when the child was born. Testing of the blood and milk showed that the nonspecific capsular swelling substance did not pass over into the milk. Specific agglutinins appeared both in the blood and milk in specimens taken after the height of the disease, but afterwards they disappeared from the milk though they remained in the blood.

### Discussion.

In the present investigation, three substances in the serum which react with pneumococci, namely, nonspecific capsular swelling substance, normal antibodies and immune antibodies, were compared. The capsular swelling substance was given most study, and the experiments done permit certain conclusions.

Nonspecific capsular swelling substance causes the best reaction, i. e. it seems to react most homologously, with *Pneumococcus* type 27, but it also reacts regularly with types 16 and 28. Some of the experiments indicate that it also affects other types of pneumococci. It causes firstly a capsular reaction, making the capsule visible in the microscope, and secondly agglutination of the pneumococci. The reaction seldom reaches the same degree or intensity as specific capsular swelling, but it is nevertheless easy to observe.

Nonspecific capsular swelling substance occurs in human serum in acute diseases such as bacterial infections, in processes accompanied by disintegration of the tissue such as myocardial infarction, and after injections of nonspecific irritants or antigens. It did not occur in the cases I examined of virus disease, nor, except for one case of hypernephroma, was it observed in the cases of tumor. It was not present in three advanced cases of pulmonary tuberculosis during the acute stages of this disease. In all the positive cases, the stimulating factor has to be of a certain intensity before the substance is formed. It does not occur in normal serum.

The following serologic properties are characteristic of the substance. Its concentration in serum is relatively low, and it seems to be more sensitive to heat in low concentration than in high. Consequently, sera must always be kept frozen if they cannot be analyzed directly after being taken.

The capsular swelling reaction does not take place if substances which precipitate calcium, like oxalate or phosphate, are added to the serum. As it reappears after recalcification, it may be assumed that the presence of calcium is necessary for a reaction to be visible in the microscope.

The substance is easily absorbed by pneumococci of types 27 and 28 and also, though not so readily, by type 16. The other pneumococcic types and other bacteria absorb it to a lesser degree.

On dialysis of serum against distilled water, the substance passes over to the water-insoluble fraction, and on electrophoresis it moves at a rate which assigns it to the serum globulin and which is more rapid than that of the  $\gamma$  fraction.

It has not been possible to produce nonspecific capsular swelling substance in horses or guinea pigs. In rabbits, however, a substance with the same properties but reacting best with type 16 can be produced.

Human serum often contains reactive substances against several types of pneumococci under normal conditions. The number of these antibodies varies from serum to serum. The content of normal antibodies in the serum is low, and these antibodies are often directed against types of pneumococci which are not common in human pathology. They react independently of the presence of calcium in the serum and are absorbed specifically when they occur in their higher concentrations. When they occur in lower concentrations, they are very labile, are easily destroyed when the serum is stored and also disappear in absorption tests with heterologous bacteria.

Horse serum contains an abundance of normal antibodies, and normal antibodies against pneumococci of types 19, 27 and 28 have been observed in guinea pig sera. Rabbit serum, on the other hand, normally seems to lack reactive substances against pneumococci.

The present investigation throws but little light on the question of the development of normal antibodies. The fact that in man they are seldom directed against the ordinary infecting types of pneumococci, and that horses which seldom have pneumococcic

infections are abundantly supplied with normal antibodies argues against their being caused by unapparent infections. On the other hand, the fact that the most common antibody of guinea pigs is directed against *Pneumococcus* type 19, which causes epizootic disease among these animals, is a point in favor of the infection theory. No doubt there exist both reactive substances which develop without homologous stimulation and antibodies which develop after unapparent infections and which have the same nature as immune antibodies.

Nonspecific capsular swelling substance resembles normal antibodies in its low content of reactive substance. The significant difference is that the capsular swelling substance is sensitive to calcium, while normal and immune antibodies react in calcium-free serum.

Absorption experiments reveal another difference. Nonspecific capsular swelling substance is absorbed by pneumococci of types 16, 27 and 28. Normal and immune antibodies can only be absorbed homologously for the most part, though overlapping reactions between the different types of pneumococci may occur.

Electrophoretic analysis shows that the capsular swelling substance probably belongs to the  $\alpha$  or  $\beta$  fraction of the globulin, while normal antibodies probably belong to its  $\gamma$  fraction.

In some experiments it was difficult to judge whether the reaction was due to the presence of normal antibodies or whether it was caused by nonspecific capsular swelling substance. Thus it happened that types 10, 18 and 23 reacted with serum while it had a high content of the nonspecific substance, but not when the concentration decreased. As these reactions were sensitive to calcium to a certain extent, there was reason to suspect that the capsular swelling substance reacted with types 10, 18 and 23 besides the ordinary types 16, 27 and 28 in this serum. This observation gives support to my original conception, that nonspecific capsular swelling substance can react with many different types of pneumococci under certain conditions. However, many of the reactions which I originally referred to the nonspecific substance have since proved to be caused by normal antibodies. Where the borderline lies between the activities of these two substances, normal antibodies and nonspecific capsular swelling substance, is difficult to determine from my results. The experiments with absorption and oxalated blood do not give any definite information.



I have not called nonspecific capsular swelling substance an antibody because it has not been possible to demonstrate a homologous antigen. The reacting types 16, 27 and 28 have heterogenetic points of contact (table 1) but no proof that even type 27, which is best adapted to the substance, contains antigen which is homologous to it has been forthcoming.

Comparison between the human nonspecific capsular swelling substance and that which is present in rabbits reveals that the two are not adapted to the same reagin but to ones which are closely related heterogenetically. Both substances are easily absorbed by type 28, the human substance also easily by type 27 and the rabbit substance by type 16. Type 27 also absorbs the rabbit substance but with some difficulty, and the same is true of type 16 and the human substance. Accordingly, we have the condition that serologic substances which occur under analogous circumstances in humans and rabbits are not adapted to identical reagins but to ones which are closely related heterogenetically.

Thus the hypothesis may be suggested that different species of animals are able to produce transient reactions in the serum in response to infection or injury to the tissue, and that these substances are adapted to different reagins in different animals.

Several other reactions occur under the same conditions as the nonspecific capsular swelling. Different methods have been used for their study and the results are far from unanimous. However, the investigations agree fairly well that in acute diseases the serum globulin increases before it is probable that any antibodies have been formed. This increase also includes the carbohydrate bound to the protein, and certain changes have also been observed in the serum albumin. Nonspecific capsular swelling substance could contribute to this increase in the globulin, but it is uncertain whether it occurs in such large quantities that its development would be manifested as a general increase in the protein. The increase in the  $\alpha$  fraction demonstrated by Blix in acute pneumonia and by Longsworth, Shedlovsky and MacInnes in febrile conditions might be caused by the nonspecific capsular swelling substance.

Among the substances which have been observed to fluctuate in acute diseases are some which agree fairly well with nonspecific capsular swelling substance.

Hjorth's X-lysin appears under the same clinical conditions as the substance, but it is not possible to make a direct compar-

ison of the lysin effect and of the precipitation which takes place in capsular swelling, and there is nothing to indicate that the two substances have any great likeness, as the protein of the X-lysin is supposed to lie on the borderline between the albumin and the globulin fractions, not being totally salted out by half saturation with ammonium sulphate.

The acute phase serum of Tillett and Francis shows practically the same behavior as nonspecific capsular swelling substance, insofar as the two have been examined similarly. Thus calcium is of importance for both substances. But they differ in protein, the acute phase protein being localized in the albumin fraction and the capsular swelling protein in the globulin fraction.

The nonspecific appearance of antifibrolysin in acute pneumonia and other diseases also resembles the appearance of the nonspecific capsular swelling substance, and the increase in the sugars bound to the serum protein which occurs in certain diseases, shows similar fluctuations to that of the nonspecific substance.

Accordingly, nonspecific capsular swelling does not entirely fit in with earlier known serologic facts, but there are far-reaching likenesses between this substance and other nonspecific substances occurring in acute disease and after stimulation with certain agents.

The rôle of these different nonspecific substances is not known, particularly their importance to the general increase in resistance to certain diseases which occurs after nonspecific stimulation. This nonspecific heightened resistance, certain forms of which Ørskov and Kauffmann call promunity, is generally considered to consist of omnicellular activation, an activation of the protoplasm (Weichardt), with an increase in the tissue immunity and an increase in the content of antibodies already present (anamnestic reaction). But little attention has been paid to the occurrence of the new humoral substances which the organism rapidly mobilizes after acute injuries, and which can be observed in nonspecific reactions.

There is no proof that the nonspecific capsular swelling substance is an antibody but it may be so, if one reckons with a homologous auto-antigen. The rapid fluctuations in the content of the substance do not, however, fit in with the ordinary course of the formation of immune antibodies.

There is another possibility. The globulin-forming apparatus may be stimulated by antigens or disintegrating tissue to give off

to the blood, after a short latent period, nonspecific reactive substances sensitive to calcium, belonging to the  $\alpha$  or  $\beta$  globulin fraction. The rapid fluctuations in the content of these substances would agree with such a concept, the formation ceasing as the stimulation disappears.

Regarding the usefulness of the nonspecific capsular swelling reaction in the clinic, the following remarks seem adequate at present.

No healthy person shows the reaction. It is but rarely observed in a person with a normal body temperature, but it is not a constant accompaniment of fever, its presence being dependent upon the origin of the latter. If the reaction is well marked and the swelling persists for a long time, the chances are that the disease will be one of long duration. Upon improvement the reaction may disappear quickly, even at a surprising rate, as when it shifts in one day from strongly positive to negative upon the removal of a post-pneumonic pleural exudate.

Just as fever does not necessarily produce a positive reaction, the occurrence of the nonspecific swelling is independent of leucocytosis, disturbed suspension stability of the blood corpuscles, and increase in the globulin of the plasma.

Among infections the substance does not seem to be produced in virus diseases, and in the common form of acute upper respiratory infection or influenza the reaction becomes positive only when a bacterial complication is developing.

In most acute bacterial infections the reaction is positive. Here its transient character is of clinical value, an unexpectedly long persistence, for instance in pneumonia, indicating the development of some complication. Its persistence in subacute bacterial endocarditis is likewise worthy of notice. Its behavior in tuberculosis is difficult to interpret at present.

The occurrence of the reaction in acute myocardial infarct seems a sensitive indication of persistent or subsiding tissue breakdown, but the general application of this point of view meets with difficulties in the case of tumor, many forms of which are not accompanied by the reaction, a case of hypernephroma being the only one in which it has been observed so far.

The reaction may be of value in differential diagnosis, as in the following instances. In heart disease with anginal pain and decompensation, a positive reaction points to cardiac infarction, and a negative one to myocardial failure. In differentiating between chronic endocarditis with valvular defect and subacute

bacterial endocarditis, a moderate content of nonspecific capsular swelling substance indicates the latter, while repeated negative reactions argue definitely against a bacterial process. In the case of acute meningitis with no bacteria in the cerebrospinal fluid, one should continue to suspect a bacterial origin if the capsular swelling reaction is positive; if it is strongly positive it is probably a question of bacterial infection of non-tuberculous nature. A negative reaction does not exclude tuberculosis, but a non-bacterial origin is more likely in such an event.

As a routine method in the clinic, the nonspecific capsular swelling reaction has the advantages of being simple to perform and easy to evaluate.

### Summary.

A substance occurs in human serum which causes capsular swelling and agglutination of pneumococci of types 16, 27 and 28 and perhaps of other types as well. It is not a normal or immune antibody and I have called it nonspecific capsular swelling substance. It is formed in acute stages of bacterial disease, in some acute diseases accompanied by disintegration of tissue and after the injection of antigens and nonspecific stimulants. A substance of the same kind was also observed in rabbit serum; it reacted regularly with *Pneumococcus* type 16 but seldom and only weakly with types 27 and 28.

The first two chapters give an account of the specificity of antigens and of the nature of antibodies, and the third chapter contains a summary of earlier investigations on nonspecific serologic reactions in acute diseases of man.

The fourth chapter contains a description of the methods used in my investigations and of the serologic properties of nonspecific capsular swelling substance. The nonspecific capsular swelling reaction is done according to the Neufeld method and the content of the substance titrated on a slide with constant amounts of serum against increasing concentrations of bacteria.

The concentration of nonspecific capsular swelling substance in serum is low in comparison to that of the immune antibodies, but of about the same magnitude as that of the normal antibodies.

The substance is gradually destroyed by heat and generally does not tolerate heating to 70 C. for half an hour. It seems to be more sensitive to heat in low concentrations than in high.

Normal antibodies against pneumococci occur to a great extent and are directed against different types of pneumococci in different sera. Normal antibodies and nonspecific capsular swelling substance may both occur in one serum and react with the same type of pneumococcus.

Nonspecific capsular swelling substance differs from normal and immune antibodies in that it causes no visible reaction in calcium-free serum, while the antibodies react independently of the presence of calcium.

Nonspecific capsular swelling substance is absorbed by pneumococci of types 16, 27 and 28, differing in this respect from normal and immune antibodies, which can only be absorbed by homologous antigen on the whole.

The substance gives a slight, temporary protection to mice against infection with pneumococci of types 27 and 28, but none against infection with type 1.

The substance belongs to the serum globulin where it is found in the  $\alpha$  or  $\beta$  fraction. Normal antibodies also belong to the globulin but occur in its  $\gamma$  fraction.

The substance may be produced experimentally in man by the injection of nonspecific stimulants like Sulfosin and by vaccination with bacterial vaccine. It manifests itself twenty-four hours after injection, reaches its highest concentration within three days and disappears within a week.

The fifth chapter contains a report of my experiments on animals. A substance resembling the nonspecific capsular swelling substance in man was produced experimentally in rabbits by the subcutaneous injection of sulphur or the intravenous injection of pneumococci of types 1 or 3. It reacted with *Pneumococcus* type 16 and, when present in high content, with type 28. It was manifested within twenty-four hours after the injection, reached its maximum within two days and disappeared again within five days. It could be absorbed with types 16, 27 and 28.

The nonspecific capsular swelling substances in man and rabbits are adapted to heterogenetically related but not identical reagents.

Normal antibodies against pneumococci were demonstrated in horses and guinea pigs, but no substance like nonspecific capsular swelling substance could be produced experimentally in them.

The sixth chapter describes the occurrence of nonspecific capsular swelling substance in different diseases. It occurred in almost every case of acute pneumonia, acute and chronic sepsis and

paratyphoid fever. It also occurred in one-third of the cases of scarlet fever examined, and in acute pulmonary tuberculosis, but not in all the chronic advanced cases, even when they showed large cavities. Acute exacerbations of the disease were generally followed by the presence of the substance, in tuberculosis as well. Acute meningitis caused by *Neisseriae* and pneumococci were also accompanied by nonspecific capsular swelling substance. The examined cases of tuberculous meningitis showed a weak reaction, if any at all, and the cases of meningitis caused by virus, including poliomyelitis, lacked nonspecific capsular swelling substance in the blood. Re-vaccination against smallpox did not cause the substance to appear in the blood.

Nonspecific capsular swelling occurred regularly in cardiac infarction but not in cardiac or vascular diseases without disintegration of tissue.

One case of hypernephroma was accompanied by nonspecific capsular swelling substance but the rest of the examined cases of tumor, carcinoma and one case of reticular cell sarcoma, were accompanied by very low concentration of the substance, if any, even in extremely malignant cases.

In the seventh chapter it is shown that the substance remains in the blood as long as the pathologic process is active, or about as long as the fever lasts. Its concentration varies rapidly with fluctuations in the course of the disease and when the disease subsides, it disappears from the blood.

The substance usually disappears from the blood when specific antibodies are formed, provided that no new disease supervenes.

The substance does not increase parallelly with elevations in the sedimentation rate or white blood count.

The eighth chapter records the investigations on the occurrence of the substance in other bodily fluids than serum.

It occurs in pleural exudate but often in lower concentration than in the blood. When bacteria are present in the exudate, the substance is often lacking there, despite its presence in the blood.

It has not been possible to demonstrate the substance in cerebrospinal fluid or human milk.

In the closing discussion, the relation of nonspecific capsular swelling substance to other similar substances is reviewed, and general problems regarding resistance and immunity are discussed. Finally, suggestions are made for the use of the nonspecific capsular swelling reaction as a clinical laboratory method.

## Appendix.

Table 34. — Hospital record numbers of the more important cases included in the clinical series and in the large experimental investigations.

The letters before the numbers denote the name of hospital or laboratory: E = St. Erik's Hospital, Ep = Stockholm's Epidemic Hospital, R = Radiumhemmet, S = Söderby Hospital, SB = State Bacteriologic Laboratory, PS = Psychiatric ward of St. Erik's Hospital, and Ö = Örebro District Hospital. PP = Private Patient.

Case No.	Record No.	Case No.	Record No.	Case No.	Record No.	Case No.	Record No.
83	E 5577/41	132	SB 7133/40	181	E 3101/41	230	E 729/39
84	E 4806/41	133	SB 7134/40	182	E 1680/39	231	E 2628/39
85	PS 56/33	134	SB 3674/41	183	E 1857/39	232	E 2799/39
86	E 5214/41	135	SB 9294/41	184	E 2813/39	233	E 8648/39
87	E 5945/41	136	SB 228/42	185	E 5504/39	234	E 1964/39
88	E 6145/41	137	SB 9675/41	186	E 6118/39	235	E 2550/39
89	E 2118/41	138	SB 8184/41	187	E 7787/39	236	E 4159/39
90	E 5269/41	139	SB 9552/41	188	E 9213/39	237	E 4632/39
91	E 3046/42	140	Ep 1906/40	189	E 9299/39	238	E 4919/39
92	PP	141	N 1031/41	190	E 502/40	239	E 2/39
93	E 5042/41	142	E 3076/39	191	E 8780/39	240	E 873/39
94	E 6084/41	143	E 3156/41	192	S 11/39	241	E 1191/39
95	E 6145/41	144	E 3647/41	193	S 38/39	242	E 2624/39
96	E 6117/41	145	Ö 409/41	194	S 392/39	243	E 3242/39
97	E 6414/41	146	PP	195	S 548/39	244	E 4329/39
98	E 7523/39	147	E 3611/41	196	S 587/39	245	E 8672/38
99	E 5327/39	148	Ep 2960/41	197	S 864/39	246	E 2143/39
100	E 2921/41	149	E 6404/39	198	S 883/39	247	E 4496/39
101	E 5093/41	150	E 7188/39	199	S 922/39	248	E 1222/39
102	E 3575/41	151	Ep 2762/41	200	S 949/39	249	E 2300/39
103	E 2935/40	152	E 3300/41	201	S 989/39	250	E 2459/39
104	E 2921/41	153	E 2832/41	202	E 7795/39	251	E 757/39
105	E 5109/41	154	Ep 2829/41	203	E 3558/40	252	E 3022/39
106	E 5276/41	155	Ep 2877/41	204	E 591/40	253	E 3884/39
107	E 2999/41	156	Ep 2889/41	205	PP	254	E 4009/39
108	E 4906/41	157	Ep 2924/41	206	E 5574/40	255	E 4036/39
109	PP	158	Ep 2960/41	207	E 1265/41	256	E 2203/39
110	E 5442/41	159	Ep 2961/41	208	E 4823/39	257	E 1899/39
111	PS 14/36	160	Ep 2770/41	209	E 2118/41	258	E 4155/39
112	—	161	Ep 2774/41	210	E 3662/42	259	E 5688/41
113	PS 12/38	162	Ep 2740/41	211	E 1331/42	260	E 3679/39
114	PS 25/28	163	Ep 2751/41	212	E 3111/41	261	E 3151/41
115	PS 30/33	164	Ep 2757/41	213	R 12818/41	262	E 6313/39
116	E 556/39	165	Ep 2814/41	214	R 12777/41	263	E 4379/39
117	E 6419/39	166	Ep 2826/41	215	R 12147/41	264	E 2768/41
118	E 7949/39	167	Ep 2791/41	216	R 12914/41	265	E 2907/41
119	E 39/40	168	Ep 2803/41	217	R 12279/41	266	E 3453/41
120	E 3184/40	169	Ep 2804/41	218	R 12524/41	267	E 6349/41
121	E 4889/39	170	Ep 2800/41	219	R 12384/41	268	E 3385/41
122	E 2386/39	171	Ep 2887/41	220	R 12291/41	269	E 4777/39
123	E 7161/39	172	E 1972/39	221	—	270	E 3483/39
124	E 3219/39	173	E 2689/39	222	E 4140/39	271	E 2770/39
125	E 1735/41	174	E 4693/39	223	E 8662/38	272	E 3101/41
126	E 2858/41	175	E 4927/39	224	E 3997/39	273	E 5093/41
127	E 4886/41	176	—	225	E 5/39	274	E 5250/41
128	E 5123/41	177	E 5832/39	226	E 1837/39	275	E 5093/41
129	SB 7003/40	178	E 6928/39	227	E 2939/39	276	E 5304/41
130	SB 7130/40	179	E 8647/39	228	E 2299/39	277	E 2624/39
131	SB 7132/40	180	E 9092/39	229	E 2935/40		

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# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CXLIII

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## PURIFICATION OF POLIOMYELITIS VIRUSES EXPERIMENTS ON MURINE AND HUMAN STRAINS

*By*

SVEN GARD

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UPPSALA 1943. ALMQVIST & WIKSELLS BOKTRYCKERI A.-B.

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FROM THE INSTITUTE OF PHYSICAL CHEMISTRY  
AND THE DEPARTMENT OF HYGIENE AND BACTERIOLOGY  
UNIVERSITY OF UPPSALA, SWEDEN

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PURIFICATION  
OF POLIOMYELITIS VIRUSES  
EXPERIMENTS ON MURINE AND  
HUMAN STRAINS

BY

SVEN GARD

UPPSALA 1943



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ALMQVIST & WIKSELLS BOKTRYCKERI A.-B.

43200

*To*  
*My Father*



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## Preface.

The poliomyelitis research at the Institute of Physical Chemistry is sponsored by "Konung Gustaf V:s 80-Årsfond". I am deeply indebted to the Board of Directors of the Fund, and particularly to Professor Henning Waldenström for entrusting me with a share in these investigations.

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Last but not least I thank Professor Carl Kling, my chief at the State Bacteriological Laboratory, Stockholm. He was my teacher and he introduced me into my present field of work. Through his unceasing scientific interest and tireless search for the truth, he has set a great example. I will always appreciate his friendship as a great honour.

Uppsala, March 1943.

*Sven Gard.*

## Introduction.

In 1909 Landsteiner and Popper (95) made the important discovery that infantile paralysis could be transferred to and maintained in passage in monkeys. In the field of experimental poliomyelitis research thereby opened, there followed a period of about thirty years, characterized by steady although slow progress. The ultimate aim of medical science is control of disease, and the research during this first epoch culminated in the elaboration of a number of therapeutic and prophylactic procedures — serum treatment, the Kolmer vaccination, zinc sulphate prophylaxis — with which great expectations were connected. When tested on a large scale in practice, however, they failed.

These failures aroused latent suspicions that the path hitherto followed might be a blind alley. Thus, in the later thirties an almost tumultuous development set in, which deserves to be termed revolution rather than evolution. The fundamentals of the prevailing theories were found at best unreliable, and characteristics of the virus-host interaction previously not recognized were now observed, necessitating a radical change of the conception of the whole problem. One of the principal reasons why the previous research had been lead astray was that most of the experimental work had been done in a heterologous host, the Rhesus monkey, and that it had not been recognized that the virus-host interaction in this animal in important respects differed from that in man.

When, about three years ago, the study of poliomyelitis was taken up at the Institute of Physical Chemistry, it was in the hope that it might prove possible to attack the problem from a new angle: to study the virus itself, its properties and reactions *in vitro*, as a basis of attempts at chemotherapy and chemoprophylaxis. *A priori* this scheme was not to be considered as only a remote possibility. As a matter of fact the giant molecules, to which class the viruses are



supposed to belong, seem to be in certain respects easier to study than molecules of a somewhat smaller order of magnitude.

It was, however, clearly realized that this program must involve extensive animal experiments as well. As a study of the virus in a model host had proved apt to lead to false conclusions, it was deemed advisable instead to study a model virus in its natural host. As model virus that of mouse encephalomyelitis (Theiler's virus) was close at hand. There must, in fact, be reason to expect that the remarkable similarities between the virus of human poliomyelitis and that of the mouse disease would not be limited to the observations already made, but that any further experience gained would be mutually relevant.

The first and fundamental item of the vast research program was the purification of the viruses in question. The present paper is a report on these experiments.

### **The group of poliomyelitis viruses.**

The avidity of a virus to certain tissues or organs and to certain host species has been a matter of much discussion. Attempts have been made to classify the viruses on the basis of their organotropism. At one end of the scale the pantropic viruses are equally capable of invading and multiplying in all kinds of tissues and organs. At the opposite end we find the monotropic viruses with affinity to one type of cells only. A similar variation is observed regarding possible hosts. Usually a virus has but one or a limited number of natural or homologous hosts. The virus-host interaction is usually conceived as an adaptation from the side of the virus to the habits of the host, guaranteeing the subsistence of the species. As a rule the virus can be experimentally transferred to and propagated in species other than the natural host, accessory or heterologous hosts. These often belong to species closely related to the natural hosts but sometimes the most whimsical constellations are observed. The combination of possible hosts is often a reliable characteristic of a given virus, by Doerr (29) called "Infektiositäts-Spektrum."

Sometimes one will find groups of closely related viruses, with the same organotropisms, causing identical symptoms and lesions, differing only with regard to host specificity. They have as a rule a narrow infectiosity spectrum. Such groups are for instance the rickettsiae, the pox and the influenza viruses.

With regard to the properties now mentioned the virus of poliomyelitis was for a long time considered almost unique. It was exquisitely neurotropic and with a narrowly limited tropism at that, directed towards certain functionally characterized elements of the central nervous system (CNS). It had an extremely narrow infectiosity spectrum, man being the only natural host and accessory hosts being found only among a very limited number of higher primates. Al-

though it was admitted that different strains of virus might exist, differing principally with regard to immunological properties, the existence of other viruses to be classified in the same group was not recognized.

This conception had, however, to be revised. Already in 1912 Kling, Pettersson and Wernstedt (88) were able to show the existence of poliomyelitis virus in the intestinal contents removed at autopsy of cases of infantile paralysis. It was not, however, until Trask, Vignec and Paul (159) in 1938 had instituted the ether-treatment, thus considerably facilitating the examination of stools, that the significance of this observation was clearly realized. It has now been confirmed by numerous investigators [for references see Trask, Paul and Vignec (158)] that large quantities of virus are regularly excreted from the intestines, not only by patients with the typical paralytic form of the disease but by abortive cases and healthy contacts as well. Virus has also been found in the intestinal wall (133), and in mesenteric lymph nodes (87). Furthermore virus could be detected in sewage from urban districts, where cases of poliomyelitis had occurred (116, 117, 85), partially under conditions to suggest that considerable quantities of virus were excreted in a population among which only a limited number of manifest cases had been observed. On the basis of this evidence one must assume that the virus is capable of multiplying not only in the central nervous system but also in the intestines.

Although experimental transmission of poliomyelitis to the ordinary laboratory animals had been without success in numerous attempts (61, 51), experiments to find new hosts have been repeated now and again. In 1939 Armstrong (1, 2) was the first to report success. In several experiments he was able to transfer the Lansing strain from monkeys to cotton rats (*Sigmodon hispidus*), and after passage through cotton rats to albinotic mice. The symptoms and histological lesions produced were indistinguishable from experimental poliomyelitis in monkeys. The virus retained its pathogenicity to monkeys even after prolonged serial passage in mice. Through neutralization and cross immunity tests the identity between the mouse passage strain and the original Lansing strain has been evidenced.

Transmission experiments of this kind can not be arbitrarily reproduced. Armstrong tested a number of strains, being successful only

with the Lansing strain. Negative results have also been reported by Toomey and Takacs (154), using 9 different strains, further by Hammon (59) and by Kramer and Mack (92), the latter authors using different procedures of inoculation known to increase the susceptibility of experimental animals to infection. Toomey and Takacs (155, 156) have later reported success with two strains of virus, after preceding treatment of the test animals with repeated subcutaneous injections of certain bacterial vaccines, a method previously used by Toomey to increase the susceptibility in monkeys to poliomyelitis.

Jungeblut and Sanders (76, 77, 78), working with the Sk strain, succeeded in producing in cotton rats an encephalitis of extremely acute course and high mortality rate. After passage through cotton rats the strain suddenly lost its pathogenicity to monkeys, producing only a subclinical infection with nondescript lesions. From cotton rats the virus could be passed to mice and maintained in serial passage, during which a certain fixation seemed to occur, the titer rising from  $10^{-6}$  to  $10^{-9}$ . At the same time the invasiveness increased, all tested routes of inoculation being equally successful. The mouse virus could easily be established in tissue culture of mouse embryo brain, the titer of the culture fluid reaching extremely high values. The fixed mouse strain produced encephalitis occasionally when inoculated into monkeys. The predominating symptoms were fever, coarse tremor, tonic-clonic convulsions and prostration. Surviving animals proved to be non-immune when tested with the original Sk strain. The mouse virus could be further transferred to guinea pigs, producing fever, paralysis of the hind legs and often death, the histological lesions resembling those of poliomyelitis. The strain could be maintained in brain to brain passages in guinea pigs but only for a limited number of generations, the infectivity gradually decreasing.

The last mentioned observations are rather confusing, differing definitely from the general experience of poliomyelitis virus on many important points. In interpreting the results one has to keep several possibilities in mind. The strain might have been inhomogeneous from the start, new components coming into preponderance when the experimental host was changed. Or latent viruses characteristic of the new hosts might have been picked up accidentally. This is known to occur rather frequently in rapid series of passages in small rodents (67, 150, 82), a procedure that Jungeblut and Sanders consider in-

strumental in establishing the infection in cotton rats. On the other hand sudden changes, the character of mutations, might occur during passage in accessory hosts, even of a pure strain of virus. Definite conclusions as to the significance of Jungeblut and Sander's observations must be postponed until further investigations (for instance filtration endpoint determinations) have revealed the true nature of their passage strain.

For identification of the virus of poliomyelitis it has been usual to rely upon the following criteria: (a) production in monkeys of the characteristic clinical picture with the typical histological lesions, (b) successful passage to another monkey, and (c) the virus should be non-infectious to the ordinary laboratory animals. The third criterion must be considered insignificant after the observations of Armstrong, and the second is of little value since it is well known that most strains are adaptable to monkeys only with difficulty or not at all. The remaining one is in fact of a secondary nature, referring exclusively to the reactions of the host. As a matter of fact it has until now been impossible to detect any direct evidence of the presence of the virus in infected tissue in the form of inclusion bodies or the like. In most virus infections reliable information about the nature of the virus can be gained through serological reactions. In the case of poliomyelitis this is hardly feasible. The true nature of the neutralization in vitro, if specific or not, has been a matter of discussion and as to other reactions they have failed completely (123).

On the other hand, the state of affairs indicated above necessitates great caution in evaluating the observations by Armstrong and Jungeblut and Sanders, since there are known to exist at least two spontaneous virus diseases in different animal species, showing all the clinical and histological characteristics of poliomyelitis. These diseases are known as the Teschen disease of swine and the spontaneous encephalomyelitis of mice (Theiler's disease).

*The Teschen disease* (Bohemian disease, infectious swine paralysis Encephalomyelitis enzootica suum) was first observed in epizootic form and recognized as a clinically and etiologically distinguished disease in the year 1929 in Teschen (Czechoslovakia). It has since then remained enzootic in this part of Europe and spread slowly southwards and westwards. In 1938 cases occurred in Bavaria and

Austria, in 1939 in Switzerland and in 1940 in the neighbourhood of Leipzig.

Klobouk (89) made an extensive study of the disease and identified the infectious agent as a filterable virus that could be propagated in serial intracerebral passage in swine. The literature on the subject is quite comprehensive and for a more exhaustive review reference might be given to articles by Lentz (98) and Weidlich (160).

Clinically an acute, a subacute, and a chronic form of the disease are distinguishable (146). The acute disease is most common in sucklings or animals just weaned. After a short stage of fever and, occasionally, of vomiting, alarming encephalitic symptoms appear: hyperirritability, sometimes coma, convulsions of different groups of muscles preferably of the head, nystagmus, and manège movements. Skin hypersensitivity is common. In most cases death occurs within 3 or 4 days.

In the subacute and chronic forms flaccid paralysis of the limbs is the predominating symptom. Sometimes spastic paralysis is observed. The hind quarters are most often involved, but isolated paralysis of other muscles has been observed. As the "simple form" Diernhofer (28) describes an ascending paralysis of the Landry type. The mortality rate is about 20 p. c. A considerable number of the surviving animals show atrophy of the paralysed muscles, but sometimes complete recovery is attained.

At autopsy no gross lesions are observed. On microscopical examination lesions are found in the CNS, consisting of ganglion cell degeneration with neuronophagia, scattered round cell infiltration and glial reaction, and finally, perivascular round cell cuffing (89, 6, 90). Meningitis is usually not observed. The lesions are confined to the gray matter, preferably of the anterior horns of the cord and the basal nuclei of the brain. According to Kment (90), however, a leptomeningitis and infiltration of the cerebellum with distinct alterations of the Purkinje cells should regularly occur.

The disease can be transferred experimentally through intracerebral injection of emulsified cord. The incubation time amounts to one to four weeks and the symptoms and lesions are identical with those of the spontaneous disease. Young animals are regularly susceptible to experimental infection, whereas a large percentage of old animals are resistant. Transmission by the nasal route is possible although with far less regularity; subcutaneous and intravenous

inoculation succeeds only occasionally. It has not been possible to transfer the disease to mice, rats, guinea pigs, rabbits, fowls, sheep, or calves (89, 28).

About the portal of entry and the excretion of the virus and the natural mode of transmission of the disease nothing is definitely known. On the basis of successful feeding experiments, Klobouk suggested the alimentary route. Diernhofer, on the other hand, advocates the nasal mucosa as portal of entry. Rats have been suspected to be intermediate hosts of the virus (28).

Several authors (136, 90, 40) have emphasized the similarities between the Teschen disease and the human poliomyelitis.

*Theiler's disease.* The first description was given by Theiler (147) in 1934. In a later communication (148) he presented details about the disease and its causative agent, shown to be a filterable virus. He emphasized in this paper the similarities between this disease of mice and the human poliomyelitis. Furthermore an extensive study of the disease and its causal agent was reported by Theiler and Gard (150, 151).

The main feature of the clinical picture is flaccid paralysis of the limbs, principally the hind legs, sometimes of the ascending type, terminating in respiratory paralysis and death. Sometimes the nervous disturbances are very mild and complete recovery is attained. More often, however, paralysis leads to muscular atrophy and contractures. With the exception of very advanced cases the animals retain their natural liveliness. Their fur is sleek, their appetite normal.

At autopsy no gross changes are found. On microscopical examination (110) lesions can be found only in the central nervous system. They consist of neuronal disintegration with the typical picture of neuronophagia, further perivascular round cell cuffing, often extending to the meninges, and a scattered round cell infiltration and glial reaction in the most heavily invaded areas. In late stages of repair nothing is to be seen except a pronounced scarcity of motor ganglion cells. Preferred areas are the anterior horns of the lumbar and cervical cord, although involvement of higher centers, especially of medulla and pons regularly occurs, at least after intracerebral infection. (Plate I.)

The attack rate of the spontaneous disease is very low. Theiler originally estimated it to one or two per thousand. Sabin and Olitsky

(132) arrived at similar figures. For all we know even this moderate figure might be too high. Olitsky (107) has later observed a series of more than 5 000 mice with no cases. The present writer observed during 30 months a total of about 15 000 animals in which period 5 spontaneous cases of the disease were discovered, four of these occurring almost simultaneously during an outbreak in the colony of mouse typhoid (caused by *S. enteritidis*).<sup>1</sup>

It is generally young mice that contract the disease spontaneously. All instances known to the present writer concern animals of from 4 to 8 weeks of age.

In contrast to the low attack rate the disease is geographically widely distributed. Besides in the U. S. it has been observed in Germany (53), in Japan (73), at the Rockefeller stations in Brazil and Uganda (Theiler pers. comm.), in Palestine (Kligler pers. comm.), and in Sweden (own observation).

The disease can be maintained indefinitely in serial intracerebral passages of brain or spinal cord. Under experimental conditions the infectious agent displays an exquisite neurotropism. Direct contact with nerve elements is the only mode of infection to yield success with any degree of regularity. With highly virulent strains of virus the intranasal and intraperitoneal routes might prove successful if very large amounts of virus are inoculated. The highest concentration of virus following infection is usually found in the spinal cord, although at the height of disease the brain and peripheral nerves might be infective as well, whereas organs and tissues outside the nervous system seem to be virus free. There is furthermore no indication of an elimination of the infectious agent from the central nervous system by way of excretion.

Recovery from spontaneous or experimentally induced disease is regularly accompanied by a solid immunity to subsequent infection. In cross immunization experiments it has been possible to show that all strains of mouse encephalomyelitis virus are very closely related,

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<sup>1</sup> This is not the place to discuss the eventual epidemiological significance of this observation. About the pathogenesis of the spontaneous infection of the central nervous system very little if anything is known. In this connection it might be mentioned that Olitsky (107) attempted to induce infection from the intestines by means of a rather drastic treatment with croton oil, resulting in almost complete loss of the mucous membranes, yet without success.



although slight immunological differences seem to exist. It has been observed that animals recovered from mouse encephalomyelitis were resistant to subsequent infection with Armstrong's Lansing strain (150).

With age the resistance to experimental infection increases although a level of immunity comparable to that attained after actual disease is never reached. Signs of increased resistance are lengthening of the incubation period, milder symptoms and lower mortality rate. Real immunity in apparently normal mice has been observed only once (own observation). In this case it was possible to isolate from the central nervous system a strain of mouse encephalomyelitis virus, clearly different from that with which the test inoculation had been performed. This unique case must therefore be regarded as one of immunity following naturally acquired infection with complete recovery.

The wild mouse (*Mus musculus*) is as susceptible to infection with mouse encephalomyelitis virus as the albinotic variant. A number of other rodents tested proved to be refractory, viz. field voles, deer mice (*Peromyscus*), white rats, guinea pigs and rabbits. It has been shown, however, that at least one strain is pathogenic for the cotton rat (150). Attempts to transfer the disease to monkeys have been without success.

The infectious agent passes ordinary bacterial filters readily. Theiler and Gard (150) determined the filtration endpoint to between 35 and 27 m $\mu$ . With exactly the same technique Theiler and Bauer (149) had previously found a filtration endpoint for human poliomyelitis virus between 35 and 30 m $\mu$ . The virus of mouse encephalomyelitis is rapidly destroyed at temperatures above 50° C, but shows a considerable stability at room and ice box temperatures. Stored in 50 p. c. glycerin at + 4° C infected brain and cord retain the infectivity for years. An aqueous solution was kept under ether in the ice box for 100 days without apparent loss of activity. Moderate concentrations of acetone are well tolerated whereas ethyl alcohol has a definite inactivating effect. The virus can be precipitated with ammonium sulfate and redissolved without loss of activity. Desiccation from the frozen state on the other hand, frequently used as a method for conservation of the viability of different viruses and bacteria, is deleterious to the virus of mouse encephalomyelitis as it is to human poliomyelitis virus (51). Exposure to the air seems to have little

influence on the stability of the virus, but oxygen in the nascent state has a definite inactivating effect.

In 1939 Olitsky (106) made the important observation that encephalomyelitis virus could be recovered from the intestinal contents of apparently healthy mice. Later studies on this subject have revealed the following facts (107, 108, 151). In sucklings the virus has never been found even if the mother was a carrier or was infected experimentally. At the age of weaning from two to four weeks, virus can occasionally be found in the intestines. From then on the frequency of positive findings rapidly increases so that at the age of six weeks practically 100 p. c. of stock mice carry the virus in their guts and excrete it with their feces. They remain carriers for shorter or longer periods of time, in some instances probably for life.

Virus can be recovered from feces, from the intestinal contents, from the intestinal wall, frequently from mesenteric lymph nodes, occasionally from the stomach contents but not from the stomach wall, never from oesophagus nor from any part of the head and not from any interior organs. The actual site of infection and multiplication of virus is not definitely known. After rinsing of the gut in running tapwater with gentle rubbing of the mucous membrane it will be found on histological examination that considerable quantities of intestinal contents are still retained in the crypts and between the villi of the mucosa. From intestinal wall thus treated, virus is easily recovered. When the guts are washed more energetically by repeated shaking with water or physiological saline solution, the mucous membrane will be severely injured, most villi completely destroyed and only the basal parts of the epithelium left. In spite of this, contents are likely to be found still clinging to remnants of the mucous membrane. Peyer's patches are usually well preserved. Such preparations have generally a low virus content and are occasionally virus free. It seems probable, therefore, that the virus is to be found either in the superficial parts of the mucous membrane or extracellularly on the surface of the epithelium. The presence of virus in the lymph nodes might be interpreted as the result of absorption rather than as evidence of invasiveness.

Furthermore Olitsky has been able to demonstrate the presence of virus in wild mice, trapped in the laboratory, but so far not in wild mice caught at some distance from places where stock mice were housed (111).

## Human, murine and porcine poliomyelitis.

Students of poliomyelitis have long been used to looking upon this virus as something absolutely unique, in a way of speaking as a gruesome privilege of the human race. From a scientific point of view this attitude is of course absurd. The existence of coordinated types of the virus with different host specificity might be denied but not until the facts have been taken into unbiased consideration. If on the other hand relationships are found, close enough to support the conception of different strains as variants or types of the same species, it would be only logical to express this view in the choice of nomenclature.

Iguchi (73) suggested the name mouse poliomyelitis for Theiler's disease, and this terminology was adopted by Olitsky. From certain quarters, however, objections have been raised against the premature use of this name, especially on behalf of the confusion brought about by Jungeblut and Sander's observations. The problem of the systematic position of Theiler's virus has undoubtedly gained in importance after the establishment of murine strains of the human virus. This fact might justify an attempt to review the present state of the question.

Of the two viruses, that might be classified with the poliomyelitis virus, that of mouse encephalomyelitis has been by far the most extensively studied and an analysis of the situation must be concerned principally with this virus. Available data on the Teschen disease quoted from the literature will however be included in the following survey, which is presented in tabular form in order to avoid reiterations. (Table I.)

A few items of the table need some further elucidation.

The seasonal distribution in poliomyelitis is very characteristic, the peak of the frequency curve almost invariably to be found in the fall. This is particularly conspicuous in the cold and temperate climates of the northern as well as the southern hemisphere. In subtropical zones the distribution is much smoother, and in the tropics seasonal differences are hardly noticeable. A similar frequency distribution of the spontaneous mouse disease has not been observed, with the possible exception mentioned on page 11, when in the course of one year a total of four cases was observed in the mouse colony in question, all of them within a period of two weeks in August and

Table I.

Comparison between human poliomyelitis, mouse encephalomyelitis, and the Teschen disease.

	Human poliomyelitis	Mouse encephalomyelitis	Teschen disease
Clinical picture	Preparalytic stage of fever and meningeal symptoms.	Not known	Fever and meningeal symptoms.
	Paralytic stage: Flaccid paralysis, mainly of the limbs.	The same	The same
	The ascending type of paralysis common.	The same	The same
	Cerebral forms exist, with ataxia and spastic symptoms.	Cerebral forms exist, tonic-clonic convulsions main symptoms.	Cerebral forms common in young animals. Spastic symptoms.
	Non-paralytic forms exist.	The same	The same
	Incubation period of experimental disease usually 10—14 days.	The same	The same
Pathology	No gross lesions.	The same	The same
	Histological lesions confined to the CNS.	The same	The same
	Primary process: neuronal disintegration, neuronophagia.	The same	The same
	Secondary features: glial reaction, round cell infiltration, perivascular cuffing.	The same	The same
	Principal site of lesions: anterior horns of the spinal cord.	The same	The same

Table I cont.

	Human poliomyelitis	Mouse encephalomyelitis	Teschen disease
Epidemiology	Annual morbidity rate about 1:10 000. Epidemic attack rate sometimes up to 10 p. c.	Attack rate about 1:5 000.	Epizootic attack rate up to 50 p. c.
	Age distribution: prevalence of children and young adults	Prevalence of young mice.	Prevalence of young animals.
	In temperate climates characteristic seasonal distribution.	Seasonal distribution even.	Cold-wet seasons preferred.
	Portal of entry of virus not definitely known, probably alimentary tract, principally intestines.	Not known. Probably intestines.	Not known
	Elimination of virus: principally feces.	The same	Not known
	True contact cases probably rare.	The same	The same
Immunology	Reinfections are very rare.	Solid immunity after recovery from spontaneous or experimental infection	The same
	Immunologically different strains of virus seem to exist.	The same	Not known
	Neutralizing substances appear in the blood with age. Apparently no connection with clinical disease.	The same	Not known
	Attempts at precipitin reaction and complement fixation unsuccessful	The same	Not made

Table I cont.

	Human poliomyelitis	Mouse encephalomyelitis	<i>Teschon disease</i>
Distribu- tion of virus	In clinically mani- fest cases: Virus in the CNS but no other tissues ex- cept the intestinal wall and sometimes mesenteric lymphno- des.  Healthy carriers har- bour virus in the intestines. To what extent not known.	The same  Carrier state is the rule.	Virus in the CNS. Intestines with con- tents not examined.  Not known
Properties of the virus	Infectiosity spect- rum narrow: man, certain primates, oc- casionally cotton rat and mouse.  Under experimental conditions strictly neurotropic.  Generally not cul- tivable in tissue cul- ture.  Filtration endpoint: 30 m $\mu$ .  Remarkable resi- stance in vitro.	Infectiosity spect- rum narrow: albin- otic mouse, gray mouse, occasionally cotton rat.  The same  The same  Filtration endpoint: 30 m $\mu$ .  The same	Ordinary laboratory animals refractory.  The same  No attempts  Not studied  Not studied

September. The domestication of the animals, however, makes a direct comparison meaningless. From a climatic point of view the domesticated mice might be said to live under conditions close to those prevailing in the tropics.

The portal of entry of the virus of human poliomyelitis is not definitely known. From experiments on Rhesus monkeys, which can be "naturally" infected only through nasal instillations, it was generally assumed that the olfactory region of the nasal mucous membrane was the main portal of entry, and that the disease was

transmitted through droplet infection. In Rhesus monkeys infected intranasally characteristic microscopic lesions are regularly produced in the olfactory bulbs (131). In human cases of poliomyelitis, however, lesions seldom occur in the olfactory region of the brain and particularly not in the olfactory bulbs (54, 60, 62, 65, 129). Howe and Bodian (70) analysed the spread of virus and distribution of lesions in the CNS after experimental infection of chimpanzees along different routes. They found that the localization of lesions often showed a characteristic pattern, owing to the mode of infection. Examining a number of human cases from this point of view (71), they found a distribution indicating that the virus had gained access to the CNS through the spinal roots, probably from the intestines, although the cutaneous route could not be excluded. In one case it was possible to locate the portal of entry to the mouth and throat. Earlier observations had called attention to the possible connection between tonsillectomy (3, 38) or tooth extraction (45) and subsequently appearing poliomyelitis, likewise pointing towards the alimentary rather than the respiratory tract as portal of entry.

These observations combined with the fact that virus can be recovered only occasionally and in small quantities from nasal washings<sup>1</sup> but almost regularly and in large quantities from stools, decidedly favour the theory that the entry point of the virus is to be found in the alimentary tract. As a further indirect evidence of the alimentary mode of transmission versus droplet infection may be mentioned that Rhesus monkeys, which succumb to intranasal but are refractory to alimentary infection never contract the disease spontaneously even if caged with experimentally infected animals, whereas spontaneous infection of chimpanzees, which are readily susceptible to infection from the alimentary tract has been observed (104). Although definite evidence in the form of details about the mechanism of invasion is still lacking, the recent development of our knowledge on this point strongly supports Kling's theory of poliomyelitis as a waterborne disease, according to which alimentary infection was postulated (84).

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<sup>1</sup> Preparations termed nasal washings in the literature have as a rule been combined nasal and throat washings. To the writer's knowledge no serious attempts have been made to avoid contamination with throat discharge. The virus found in preparations of that kind must, therefore, not necessarily originate from the nasal mucous membrane.

In the case of mouse encephalomyelitis the present data tend to show that an apparently symptomless infection of the intestines is the normal course of the disease, while the "typical" paralytic form has to be regarded as a rare accident. Since mice are habitually coprophageous, it is natural to assume alimentary transmission of the infection. Attempts to establish enteric infection and carrier state in sucklings by feeding with active virus have failed, however. Sometimes the mice thus treated succumb in an invasion of the CNS, while in the remainder the virus is rapidly eliminated from the intestines, usually in the course of 24 hours. In suckling mice, therefore, some condition necessary for the development of carrier state, even temporary, seems not to be fulfilled. It is close at hand to connect the act of weaning and the changes of the intestinal bacterial flora, thereby induced, with the apparent change in disposition to enteric infection. On this point, however, no significant observations have been made so far.

As to the mechanism of invasion of the CNS and the point of entry nothing is definitely known. One case was examined from this point of view by the present writer. In serial sections of the CNS it was not possible to detect any lesions in the olfactory bulbs or at any level above the oblongata. In analogy with the observations by Howe and Bodian this would indicate that the virus had gained access to the CNS through the spinal roots, pointing towards the intestines as portal of entry.

The theory of transmission of poliomyelitis through personal contact was previously generally accepted and is still advocated in spite of the fact that secondary cases explainable through direct contact are extremely rare. One had therefore to assume indirect contact through symptom-free intermediate carriers as the normal mode of transmission. The comparatively rare institutional and family outbreaks of the disease were usually quoted as instances of direct contact infection. The time interval between family cases is generally less than a week, which fact led to the assumption of a very short incubation period, hardly in line with the experience from experimental poliomyelitis. An analysis of some institutional and family epidemics in Sweden showed, however, that these could be reasonably explained as cases of simultaneous infection with variation in the length of the incubation period within the limits usually observed in experimental poliomyelitis, another indirect evidence of alimentary transmission (45).



The neutralization reaction in poliomyelitis has been subjected to extensive study. The comprehensive literature in this field will not be reviewed in this connection; the experimental results only may be summarized as follows. The frequency of positive neutralization reaction increases with age. The frequency of positive reactions in convalescents is usually slightly below that of normals of the same age. The neutralizing titer of pooled convalescent serum is usually lower than that of pooled normal serum. A positive reaction affords no reliable protection against infection. Trask and Paul actually describe a case, where the reaction was positive on the third day of illness and after three weeks, but negative on a later occasion (157). There is, thus, little evidence to show any connection between the typical disease and the appearance of neutralizing substances in the blood. Whether this process is to be regarded as an expression of developing maturity resistance ("autarcesis", Aycock) or as the only visible response to repeated enteric infections cannot be settled at present.

Regarding mouse encephalomyelitis Olitsky has shown that serum from normal adult mice is capable of neutralizing the virus, and that no differences in this respect could be demonstrated between normal and convalescent serum (109).

\*     \*     \*

From the data in table I it is evident that the viruses of human poliomyelitis and mouse encephalomyelitis are closely related. In the writer's opinion particular weight should be attached to the identity of host reaction against infection and to the identity of particle size of the viruses.

Typical neuronophagias are considered pathognomonic for poliomyelitis. They are seldom observed in other virus infections of the CNS but they are always present in poliomyelitis, at least in the early stages of the disease. The micrographs of plate I reveal the remarkable similarity of histopathology in mouse encephalomyelitis and experimental poliomyelitis in monkeys.

This feature refers, however, primarily to the host. More significance as evidence of a true relationship must be attached to the results of the filtration endpoint determinations. With the technique introduced by Elford (32, 34) easily reproducible results can be ob-

tained. The particle size of a certain virus seems to be a fairly constant quality, at least no appreciable variation can be detected by present methods. The filtration endpoint must therefore be regarded as an important characteristic of a virus. For instance two viruses with similar clinical manifestations as those of vesicular stomatitis and of foot-and-mouth disease can easily be distinguished by means of ultrafiltration, one having an endpoint of 120, the other of 25  $\mu$ .

In a filtration experiment on poliomyelitis virus Theiler and Bauer (149) found that the virus passed a 35  $\mu$  membrane in one out of 4 tests, but had negative results with a 30  $\mu$  membrane in 6 tests. Elford, Galloway and Perdrau (35) found that the virus passed readily 40  $\mu$  membranes, and in one instance out of 5 tests a 27  $\mu$  membrane filtrate apparently produced immunity in the test animal, although no clinical infection was obtained. As positive evidence this result must be considered questionable and 30  $\mu$  as retaining pore diameter would be a safer estimate than the value of 25  $\mu$ , assumed by Elford. As already mentioned, Theiler and Gard (150) found that the mouse encephalomyelitis virus readily passed 35  $\mu$  membranes, but was retained by a 27  $\mu$  membrane. In this case, too, an estimate of 30  $\mu$  for the filtration endpoint is reasonable. According to Elford's formula the particle diameter of both viruses can be calculated to 10 to 15  $\mu$  (33).

In the range of filtration endpoints between 25 and 35  $\mu$  no other neurotropic viruses are represented, and only one additional animal virus, viz. that of foot-and-mouth disease. Under these circumstances identity of particle size must be considered as strong evidence of close relationship.

All facts considered, there remains in the writer's opinion no essential distinguishing differences between the viruses discussed except the species specificity. As the situation is, they must be conceived as variants or types of the same species until evidence to the contrary can be produced. Consequently they should be classified together and uniformly denominated. From several points of view the name poliomyelitis virus is most unsatisfactory. It is, however, established by usage, and until the question of virological nomenclature has been brought out of its present state of confusion, this name has to be accepted. In the following, therefore, the respective diseases will be called human, murine or mouse, and porcine or swine poliomyelitis,

and the causal agents referred to as poliomyelitis viruses of the human, murine, and porcine types.

The experiments to be reported were to a certain extent based upon this conception as a working hypothesis. In this study the mouse disease and its virus served as model of the human poliomyelitis. All methods were accordingly worked out in detail in experiments on the mouse virus and then simply applied to human strains. As will be shown in the following this simplification was justified by the results.

It seems to be very important to begin the specific treatment at the earliest possible moment and to give fairly large doses of antibody immediately.

For Patient No. 2 the diagnosis was made very quickly and a vigorous specific treatment was commenced only 24 hours after the onset of the disease. Complete cure was obtained in a very short time. However, one contributory factor was possibly the circumstance that the patient was a relatively old child (5 years). As already stated, prognosis is best for older children.

While the treatment is in progress the temperature curve will be of only little guidance. In the case of one of those who died (No. 5) there was an initial fall in the temperature, decreasing cell count and transient improvement. As the patient was only 7 months old, was very ill and the onset of the disease was at least six days before, a more intensive treatment ought to have been instituted from the start, but this was impracticable owing to lack of serum. The rapidly falling titre for free antibody in the blood shows that the treatment was insufficient. It is also possible that serum should have been administered intraspinally.

In respect of Patient No. 4, for whom the bacteriological diagnosis was made somewhat late owing to the slow growth of the bacteria, a distinct improvement was observed in the general condition under the sulphonamide and penicillin treatment, the temperature became normal and the pleocytosis subsided, although bacteria were still found on cultivation. However there was a rapid relapse, and a quick cure was only effected after the application of a large quantity of antibody.

On the other hand, a persistent temperature increase may be a result either of the sulphonamide therapy (drug fever) or the administration of antibody (serum reaction). This was undoubtedly the case with Patient No. 1 and probably also Patient No. 6. In the latter instance, however, we considered it necessary to prolong the treatment because bacteria appeared twice in the spinal fluid after it had been sterile. In all the patient was given 770 ml serum = 1,071 mg specific antibody-N divided over 14 injections spread over a period of 33 days. Neither in this nor in the other cases did we observe symptoms of any gravity attributable to the administration of non-specific protein. As a general rule, however, it will undoubtedly be better to apply large quantities of serum at the very beginning rather than continue administration over such a long time.

monkey poliomyelitis. It is usually observed only in animals infected with a medium amount of virus. During this stage the maximum concentration of virus in the brain is not yet attained.

The second stage is characterized by temporary attacks of tonic-clonic convulsions. These fits are usually provoked by mechanical, acoustical or optical irritaments but sometimes they seem to appear spontaneously. Spinning the animal by the tail seems to be a reliable way to incite them. They are usually of short duration, about 30 seconds. After an attack a refractory period sets in, during which it is impossible to incite a new fit. A typical attack begins with twitches of the eyelids and jerks of the head, the back is stretched, sometimes the animals turn over backwards, and characteristic clonic movements of the fore limbs set in. In laboratory slang this condition is called "jitter-bug disease," which is an excellent description of the behaviour of the animals. (Plate I) Sometimes the mere contact with the floor incites spastic movements of the legs and the animals make a series of grasshopper-like jumps of considerable height and length. A fit often terminates in a tonic convulsion, during which the back is extremely stretched, the fore legs pressed against the chest and the hind legs stretched backwards. Respiration temporarily ceases and sometimes death occurs.

If the animal survives the early attacks, the third stage enters. Symptoms are now permanent. The animals look obviously sick. The fur is ruffled. The back is hunched. Their appetite is lost. Motor disturbances are common: convulsions, spastic or flaccid paralysis. In the males permanent erection is observed almost invariably.

Sometimes, preferably in old animals, no encephalitic symptoms appear. Then the disease takes the classical course with flaccid paralysis as the main symptom. In this case, however, the fore legs are more often involved than the hind legs, and the general condition of the animals usually more affected than in the classical disease.

The mortality rate is practically 100 p. c. Sometimes, however, old animals survive an experimental infection.

The clinical picture is to a certain extent dependent upon the route of infection. (a) After intracerebral inoculation the incubation period varies with the amount of virus injected from 2 to 20 days. The course of the disease is as described above, encephalitis being the principal feature. (b) Infection by the intranasal route is possible, when large amounts of virus are used. After an almost constant

incubation period of 15 days, the animals develop an encephalitis. The mortality rate among animals showing symptoms is practically 100 p. c. (c) After intraperitoneal inoculation of large amounts of virus a certain percentage of the animals show the classical symptoms, i. e. flaccid paralysis, usually of the ascending type. Encephalitic symptoms are rare. The mortality rate is definitely lower, usually about 50 p. c. The incubation period is constant and about 10 days. — The susceptibility of the mouse to infection by the intracerebral route is about 100 000 times as high as by the intranasal and about 10 million times as high as by the intraperitoneal route.

The lesions are identical with those of the classical disease: disintegration of ganglion cells with neuronophagia, glial reaction and round cell infiltration, mainly perivascularly. The most intense reaction is found in the basal parts of the brain and the brain stem, the cortex is regularly affected, although less intensely. (Plate I) In the hyperacute cases usually no lesions are found in the cord. Cases of longer duration, however, show typical lesions in the anterior horns.

The maximum concentration of virus is found where the lesions are most pronounced, i. e. in the brain. During the second stage of the disease the brain is regularly infective in the concentration  $10^{-7}$ , when inoculated intracerebrally in 0.02 ml amounts. The infectivity of the spinal cord is definitely lower, usually by two or three powers of ten, except after intraperitoneal inoculation when the same maximum concentration is reached.

In ultrafiltration and cross immunization experiments the FA strain has been identified by Theiler and Gard as a strain of mouse poliomyelitis virus.

When used in the experiments to be described in the sequel the strain had been maintained in brain to brain passages through more than 100 generations. As a rule each experiment was set up with a fresh passage of virus, the entire series of experiments, therefore, being extended over further about 50 passages. No appreciable changes of the virulence or any other characteristics of the strain were observed during the period in question.

In a few experiments the less virulent strain UF I was used. This strain originated from an apparently healthy mouse, 6 weeks of age and was isolated from the ether-treated intestinal contents of the animal. It has been maintained in cord to brain passages. After

intracerebral inoculations of 0.02 ml of a 10 p. c. cord suspension, the attack rate has been almost invariably 100 p. c., and the incubation period between 10 and 15 days. The clinical picture has always been of the classical type without any signs of encephalitis. The histological lesions produced have been typical.

No experiments on passage strains of human poliomyelitis were carried out.

### Sources of virus.

In several cases allantoic membranes, amniotic fluid or tissue from infected chick embryos has served as an excellent starting material in purification experiments on account of their high virus content. As will be reported later in detail, it is possible to propagate the mouse poliomyelitis virus in the growing chick. The infectivity of the embryonic tissue remains, however, on a very moderate level. For purification purposes, therefore, this source of material offers no advantages.

Tissue culture fluid, being practically free from coarse tissue debris and high molecular proteins, would be an ideal starting material. In attempts to cultivate human poliomyelitis virus on chick embryo brain tissue culture, success was reported by Gildemeister (52) and later confirmingly by Pauli (118). Sabin and Olitsky (130) were unable to confirm Gildemeister's observation but found that their strain of virus could be propagated in tissue culture of human embryo brain. Recently Jungeblut and Sanders (76) found the murine Sk strain easily cultivable on mouse embryo brain tissue culture.

In spite of previous failures by the present author (unpublished data), numerous experiments were set up in attempts to establish mouse poliomyelitis virus in tissue culture. As all efforts towards that end remained unsuccessful the experiments will be only briefly summarized. The tissues tested were mouse embryo brain, whole mouse embryo, chick embryo brain and whole chick. The culture fluid consisted of Tyrode's solution, with or without serum. Human, monkey and mouse serum as well as serum ultra filtrate (135) were used. The cultures were incubated at 37 and 18° C, aerobically as well as anaerobically. The virus remained active in these cultures for considerable periods of time, especially at room temperature. In no instance, however, an increase in activity could be detected. When subcultures

were made at intervals of five or ten days, the virus could seldom be traced beyond the fifth subculture.

The choice of source of virus was, therefore, restricted to infected brain and cord, or feces. In the course of the investigation observations were made, suggesting the possibility that differences might exist between virus isolated from the CNS and virus from the intestines of the same host. Until this question has been definitely settled, it seems reasonable to make a distinction between virus preparations derived from different sources. It must be pointed out, however, that the expressions neurovirus and intestinal virus, which will be employed in the sequel, merely indicate the origin of the virus but do not necessarily imply fundamental differences.

In purification experiments on neurovirus from mice usually batches of 500 brains were prepared. The animals were killed with chloroform as soon as definite symptoms had appeared, the brains removed aseptically and stored in 50 p. c. glycerin in the cold until used. For collection of feces the animals were caged on sifted sawdust in large boxes. Every two or three days the litter was collected and resifted under gentle rubbing and pressure. Practically only feces remained on the sieve, while sawdust, crumbs of bread and the like passed the meshes.

Purification experiments on human virus were likewise carried out on nervous tissue and intestinal contents. At a number of autopsies on typical cases of poliomyelitis, brain and spinal cord were removed. Attempts to maintain bacteriological sterility were not made. The material collected was stored in 50 p. c. glycerin in the cold until used. Brain and spinal cord from one lethal case of ventricle cancer and from a man who died from injuries suffered in an accident served as control material.

As source of intestinal virus colon washings and clysters were used. The material originated from hospitalized typical cases of poliomyelitis. Washings of from one to two liters were collected, sent to the laboratory, and treated immediately. The samples were taken preferably during the first two weeks after onset of the disease. "Normal" controls, colon washings from a number of cases without symptoms from the central nervous system, were obtained from the Medical School Hospital of the University of Upsala. Finally samples of feces from a number of healthy contacts were secured.

In an appendix on page 163 the hospital record numbers of all cases included in this study are filed.



### **Inoculation technique and diagnosis.**

In the infection experiments the intracerebral route was used throughout. The test material, consisting of different fractions from the purification process, was usually not bacteriologically sterile. On account of unavoidable losses of activity filtration could not be performed. On some occasions the solutions were treated with ether before inoculation but when this was not practicable even such precautions had to be omitted and the material was inoculated without further treatment. In spite of this, intercurrent infections to be ascribed to bacterial contamination of the inoculum were never encountered.

All inoculations were carried out under ether anesthesia. For anesthetization of mice the following simple device was employed. From a gasometer air was bubbled through a flask containing water and ether, and immersed in a waterbath of 20 to 25° C. From this flask the air was led to the bottom of a glass jar with an inserted wire net bottom, on which the animals were kept. By means of this arrangement it was possible to apply a mild and even anesthesia simultaneously to a large number of mice.

For the inoculation of mice tuberculin syringes and 20 gauge needles were used. Without previous shaving or surface disinfection of the skin the needle was inserted through the right parietal bone, and an inoculum of 0.02 ml deposited at a depth of about 3 mm below the brain surface. On monkeys the skin was shaved and disinfected with alcohol over the left temporal bone, a slit made with a knife and the bone trepanned, the 12 gauge needle of a syringe inserted, and an inoculum of 0.5 ml deposited at a depth of about 1 cm in the temporal lobe.

The diagnosis of mouse poliomyelitis was based principally upon the clinical manifestations. In tests of crucial character, however, sections were prepared and examined microscopically. In the monkeys the rectal temperature was recorded in daily measurements and the motility carefully controlled while the animals were being exercised in a special large cage. In case of typical symptoms the animals were sacrificed after the fall of the temperature, and autopsy performed. Sections of the CNS were regularly prepared and examined microscopically. As a rule control of bacteriological sterility was carried out only when the presence of gross lesions suggested bacterial infection.

### Stock animals.

Already now it may be pointed out that in the kind of experiments to be reported the breed of test animals used is of the utmost importance for the uniformity of the results. This was clearly realized from the start, and attempts were made to secure a pure breed of mice. No such animals were, however, available. There was finally obtained a mixed breed, descent not known, but very uniform of appearance and size and in good condition. This stock has since been maintained through inbreeding.

As the possible presence among the test animals of latent virus infections, that might interfere with the experimental results, is a factor of great importance, several attempts were made to clear up the situation in this respect. Various methods of provocation were tested now and again with special attention to the viruses of choriomeningitis, ectromelia and mouse influenza. No latent infections could be discovered.

Each summer, however, beginning in July or August and extending over September and part of October, a sharp increase in the mortality rate of the stock animals was observed, concerning principally sucklings and females during the first two weeks after delivery. Sometimes the animals were found dead without having previously shown any signs of disease. As a rule, however, they were in a bad condition for one or two days preceding death, with loss of appetite and emaciation. With the exception of an occasional diarrhoea usually no local symptoms were observed. Sometimes, however, convulsions and spastic or flaccid paralysis occurred, not unlike symptoms of mouse poliomyelitis. Gross lesions when found at autopsy, were restricted to the skeleton, and consisted of a certain porosity of the bones and frequently proliferative excrescences of the ribs and the vertebrae. Numerous sections of the CNS were carefully examined for the presence of poliomyelitic lesions. Now and again degeneration of ganglion cells in the spinal cord was observed but never any typical lesions. Passage experiments with various organs employing different routes of inoculation remained unsuccessful. Aerobic and anerobic cultures in plain broth and on blood agar from spleen, liver and lymph nodes were consistently negative.

Animals of this stock were sold to two other laboratories and used for breeding purposes. In none of these places the same calamity

was encountered. Although no dietary changes were instituted the disease was finally interpreted as an acute vitamine deficiency, possibly on the basis of gastro-intestinal disturbances.

During that part of the year when the conditions just described were prevalent in the animal rooms, experimental work became hazardous, partly because the numerous deaths among the test animals could not always be accounted for and partly because the mice during that period showed an increased resistance against infection with poliomyelitis. Even routine passages of the less virulent strains, were almost impossible to carry through. Under these conditions it was found necessary to discontinue the mouse experiments during the months of August through October.

On account of the international situation the supply of monkeys was very limited. Infectiosity tests could not, therefore, be carried out to the desirable extent, and titrations were out of question. Before being used the animals were subjected to a tuberculin test and their rectal temperature recorded during at least one week.

## CHAPTER III.

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### Measurement of virus activity.

Many of the properties of viruses can be studied only indirectly by determining the effect of various treatments on the infectivity. Thus, it is obvious that advances in the knowledge of viruses is largely dependent upon the accuracy of the methods with which the activity is to be measured. It was, therefore, deemed necessary to pay special attention to questions connected with this problem.

### Review of methods.

When the content of living bacteria of a given material is to be estimated, either one of two methods can be used, the plate count or the dilution method. There are advantages and disadvantages connected with both methods. The interpretation and evaluation of experimental data has been subjected to statistical analysis by several authors (56, 58, 93, 102, 124, 145).

In determining the activity of a given virus material one must make a distinction between the number of virus particles and the virulence of the strain of virus. Unfortunately this has not always been the case. From some experiments in the literature it is impossible to decide, whether an observed "attenuation" of a virus is due to a decrease in titer or to be ascribed to a real change in the virus itself. In this chapter only the enumeration of active virus particles is considered.

By analogy with the bacterial count two different procedures for particle count of viruses have been developed. The "local lesion" titration corresponds to the plate count method. This procedure was originally introduced into plant virology by Holmes (64), who found that certain species of tobacco plants produced discrete lesions in-

stead of systemic infection, when their leaves were rubbed with mosaic virus containing plant juice. The number of lesions was dependent upon the virus content of the sap. Holmes compared the local lesions with bacterial colonies on an agar plate. The lesions no doubt represent virus colonies, but as a method of particle count Holmes' procedure differs fundamentally from the plate count. The surface of a tobacco leaf has only a limited number of entry points for the virus, mainly hair cells, which endure the mechanical injury necessary for letting the virus enter, and yet survive, thus permitting the multiplication of the virus. On the other hand each entry point can give rise to only one lesion regardless of the number of virus particles that actually enter. On the basis of these assumptions Youden, Beale and Guthrie (166) have derived a formula for the relationship between number of lesions and actual virus content of the inoculum. This formula is

$$y = N(1 - e^{-\alpha x})$$

where  $y$  is the probable number of lesions,  $N$  the maximum number of lesions, i. e. the total number of entry points,  $x$  the virus concentration,  $\alpha$  a constant and  $e$  the base of natural logarithms.

It is thus evident that Holmes' method does not permit the evaluation of the actual number of virus particles present in a given solution. The titer value is only relative and must be compared with a standard. But as a method of evaluating the relative virus content it has been developed to great accuracy by Samuel and Bald (134) and by Youden and Beale (165).

In animal virology a similar method has been based on the discovery by Woodruff and Goodpasture (161) that the fowl-pox virus could grow on the chorioallantoic membrane of the developing chick, thereby producing easily recognizable proliferative discrete lesions. Since 1931, when this paper was published, a large number of viruses have been found capable of propagating on the chorioallantois and the technique has been used by Burnet, Keogh and Lush (22) for titration purposes, especially in connection with immunological studies. When proper precautions are taken the method seems to be very sensitive and yield results of great accuracy. So far no statistical analysis of the membrane technique has been published, but it must be considered probable that the analogy to the plate count is more strict than in the case of Holmes' method. From a

It being the general rule that the few streptomycin-resistant bacteria in an otherwise sensitive culture are sensitive to sulphonamides, treatment with agents of this group will slow down the rate of multiplication of the streptomycin-resistant bacteria and thus possibly prevent the development of resistant strains.

Working on this theory Alexander & Leidy (1947) (4) tried treating the severe cases of meningitis with streptomycin and sulphadiazine alone. So far, however, the material is only small.

In their latest work (4) these two authors advise the administration of streptomycin in doses of 44 mg per day kg body weight. The injections should be given intramuscularly every three hours, in addition to once a day intrathecally, 25 mg for children under three years, 50 mg for older ones. The first two injections may be given with an interval of 12 hours if necessary. To avoid the serious toxic symptoms, the streptomycin treatment should not be continued longer than four or five days. What is not achieved by this treatment will scarcely be achieved by longer dosing.

It might have been anticipated that with the intensive treating of Pfeiffer meningitis many of the serious cases which now survive would manifest signs of deep, irreparable cortical changes and hydrocephalus. To judge from the literature, this is not the case, even if there are isolated descriptions of such after-effects.

### Own Observations.

As the combined sulphonamide-serum treatment led to a decided improvement of the prognosis for Pfeiffer meningitis in Denmark also (Brochner-Mortensen, Engbæk & Schmith, 1947) (7), it was not considered advisable, having regard to the former very grave prognosis and the relatively small number of patients, to abandon this therapy for treatment with streptomycin alone, experience of this being only small as yet, judging by the literature.

Now that streptomycin has become available in Denmark, it has been recommended that for the present all patients should as far as possible be treated with streptomycin as well as sulphonamide and type-specific rabbit immune serum.

Up to May 1st, 1947, this combined treatment was given to a total of seven patients from all of whom Pfeiffer's bacillus type b was cultivated from the spinal fluid.

The principles governing the serum and sulphonamide treatment are the same as those described in the above-mentioned work.

quency figures but from their accumulated sums. The result of this operation is a weighted mean and thus more reliable.

The accuracy of the 50 p. c. endpoint titration method has been tested by Horsfall (66) on influenza virus. Using Reed and Muench's modification he found a mean deviation of  $\pm 0.17$  expressed in terms of log dilution, when the test groups comprised five animals.

It is obvious that statistical methods and formulas, developed for the purpose of evaluation of bacterial counts, are applicable to any suspension of particulate material in a fluid and thus must hold true also for virus suspensions. The formula

$$P = 1 - e^{-ax} \quad (1)$$

for calculation of the probability of takes,  $P$ , at any given concentration of virus,  $x$ , when amounts of the volume  $a$  are being inoculated, has been experimentally tested by Parker with vaccinia virus (112). This author found a fair agreement between the actual rate of takes and the calculated values. We will return later to Parker's experiments.

In most cases of experimental virus infections the incubation period, i.e. the time interval between inoculation and appearance of symptoms, is an almost constant quantity. Sometimes, however, the length of the incubation period is largely dependent upon the amount of virus inoculated. It seems logical to attempt to use this additional information about the quantity of virus inoculated for titration purposes. Bryan and Beard (17, 18) found a linear relationship between log dilution and incubation period in virus induced rabbit papilloma. This relationship can be expressed in the following formula

$$t_1 - t_2 = b (\log D_1 - \log D_2) \quad (2)$$

where  $t_1$  and  $t_2$  are incubation periods corresponding to two different dilutions of the same material with the dilution factors  $D_1$  and  $D_2$  resp. They found that the slope of this line measured by the factor  $b$  was constant regardless of the origin of the virus or the resistance of the rabbits. Comparing the titration curves obtained with the same virus preparation but in animals of different susceptibility, they found that the two straight lines ran parallel but at a certain distance from each other. These observations led to the following titration procedure. In a series of experiments a standard curve was obtained,

from which the factor  $b$  could be calculated. In an experiment with purified virus this standard curve was fitted to the 50 p. c. endpoint titer and both correlated to the protein content of the preparation. In an actual titration the test material is inoculated into two or more animals, which at the same time receive a known amount of a standard virus. The incubation times are read off and the mean difference between test and standard calculated. From the standard curve the corresponding difference in activity can be computed and the result expressed in terms of MID or mg virus protein per unit volume.

The present writer (47) has described a similar procedure for titration of certain strains of mouse poliomyelitis virus. In this case, however, there exists a linear relationship between the reciprocals of the incubation periods and log dilution. Thus, the incubation time formula is

$$1/t_1 - 1/t_2 = b (\log D_2 - \log D_1) \quad (3)$$

The factor  $b$  is not constant but varies with the resistance of the animals. Construction of a standard curve was, therefore, not feasible. There exists, however, a definite relationship between incubation time and 50 p. c. endpoint titer, that can be expressed as follows:

$$\log a = 1/bt - c \quad (4)$$

when  $a$  is the number of MID inoculated,  $b$  the above mentioned factor,  $t$  the incubation time. From several experiments with different virus material  $c$  was determined to approximately 1. A titration is done as follows. Suppose there are several tests to be compared with a standard. From the standard a series of five ten-fold dilutions is prepared, from each test two different dilutions. With each dilution a group of five or ten mice is inoculated intracerebrally with a standard amount (0.02—0.03 ml.) In each group the harmonic mean of the individual incubation periods is calculated. On the basis of the data obtained with the standard material a standard curve is constructed. Through interpolation in this curve the relative titers of the tests can be evaluated.

About the two last-mentioned methods the following remarks may be made. The appearance of symptoms seems to coincide with the maximum concentration of virus in the infected tissue. This



critical concentration is remarkably constant for a given virus and a given host. Probably only a limited number of virus particles can be produced in each susceptible cell. When this stage has been reached the cell ceases to function normally or disintegrates, and when the number of injured cells has extended sufficiently, clinically manifest disturbances appear. The regularity of the dependence of the incubation period upon the dose of inoculum, as demonstrated in the formulas (2) and (3), makes the assumption probable that the rate of multiplication of virus in the infected tissue, too, is a phenomenon of great regularity that could be expressed mathematically. Let us assume that one single virus particle for the multiplication to the critical concentration needs the time  $t$ . Let further  $y$  be the virus concentration at the time  $x$  after inoculation. Then  $(t-x)$  should represent the incubation time in the case of  $y$  particles being inoculated. In the case (2) we have

$$t - (t - x) = b (\log y - \log 1) \quad (5a)$$

as  $\log$  concentration corresponds to  $-\log$  dilution. This leads to

$$y = e^{mx} \quad (5)$$

where  $m$  is a constant. This is exactly the same function that is valid for a growing bacterial population in the logarithmic phase, and it means that each virus particle at constant intervals gives rise to a fixed number of new particles. To the writer's knowledge no attempts have been made to verify this formula by direct observations.

In the case of mouse poliomyelitis we have

$$1/(t-x) - 1/t = b (\log y - \log 1) \quad (6a)$$

or

$$y = e^{mx/(t-x)}. \quad (6)$$

In this formula the exponent contains the variable factor  $m/(t-x)$ , which increases with growing  $x$ . The rate of growth, therefore, should be "overlogarithmic" and the process of multiplication represent an autocatalytic phenomenon.

It is difficult to visualize this autocatalytic process. Probably the generation time of new particles is decreasing with growing

concentration of virus, perhaps on account of a gradual disappearance of some inhibiting influence. The latter assumption might explain the difference in the influence of resistance on the factor  $b$  in cases (2) and (3). Resistance to papilloma infection causes only a parallel displacement of the titration curve, which means that either the number of entry points, i.e. susceptible cells, is smaller, or else a certain number of virus particles are destroyed before having an opportunity to come in contact with their substrate. Once in the cells they are no longer subjected to inhibitory influence and the rate of growth remains unaffected. The resistance to mouse poliomyelitis on the other hand should partially consist of an increase of the normally existing tendency to inhibition of the rate of growth. Hence the effect on the shape of the titration curve.

### The membrane method.

As the chorioallantois technique seems to be simple as well as accurate, it was decided to test this possibility in spite of the fact that several attempts by various authors to propagate human as well as mouse poliomyelitis virus in the developing chick have given entirely negative results. /Burnet (20), Gavrilov and Fester (51), Theiler (pers. comm.)/.

By inoculation of the eggs the following simple technique was employed. After surface disinfection with alcohol the shell was punctured with a needle, the point of which was blunted so as not to injure the membranes. Two punctures were made, one over the air sac, the other over the embryo. Through suction the air sac was emptied and a new artificial air space produced over the embryo. In the transilluminator it was controlled that the chorioallantois had parted from the shell. The fine needle-point of a tuberculin syringe was inserted through the puncture into the artificial air sac and the material inoculated, the dose of inoculum being 0.05 ml. The openings in the shell were sealed with paraffin and the egg returned to the incubator. The eggs were incubated at 37° C before as well as after inoculation.

*Experiment 1.* In the first series of experiments the eggs were inoculated after 10—12 days' incubation, i.e. at an age when the specific lesions of the chorioallantois are most easily produced (21). Series of ten eggs were inoculated

with a bacteriologically sterile 10 p. c. suspension of infected mouse brain in physiological saline. At different intervals after inoculation two eggs were opened and the membranes and embryos examined. Lesions of the unspecific type were frequently found on the membranes from one to four days after inoculation. No specific proliferations were observed and no gross lesions in the embryo. Sections were made and examined microscopically. No changes of the specific type were found. Part of the membranes and embryos were ground with sterile sand and saline and inoculated intracerebrally into mice. All test animals were observed during four weeks without showing any symptoms and when tested for immunity proved to be not immune. A few eggs were allowed to hatch in due time. The chickens were quite normal. The results were so far completely negative.

Since, however, Kligler and Bernkopf (83) were able to propagate rabies virus in the chick embryo by using very young embryos for inoculation this technique was tested as well.

*Experiment 2.* Using the FA strain of mouse poliomyelitis virus the following experiment was set up. A series of ten eggs was inoculated by twos at the age of 4, 5, 6, 7, and 8 days. The embryos in the first pair both died on the day after inoculation, the remainder were all alive at the termination of the experiment. Of each pair one egg was opened on the fifth, the other on the tenth day after inoculation. Membranes and embryos were examined as usual. No gross lesions were observed. The embryos were decapitated and the vertebral column removed. Membranes, CNS, and corpus of the embryo were ground separately with sand and saline and inoculated intracerebrally into groups of five mice. The animals were observed during four weeks and surviving mice tested for immunity. The result of the activity tests is summarized in table II.

The result is very clearcut. In no instance virus could be discovered in the eggs five days after inoculation whereas ten days after inoculation the CNS was infective in embryos inoculated at the age of five, six and seven days but not in eggs inoculated at eight days of age. In no instance virus was discovered in membranes nor in the corpus minus the CNS.

In three more experiments of this kind essentially the same results were obtained. Inoculation at the age of 4 days generally caused premature death of the embryo, whereas attempts to infect 7 days old eggs in two instances were without success while in two instances comparatively small quantities of virus were present in the CNS.

*Experiment 3.* A passage experiment was performed as follows. Infective CNS from an embryo inoculated at the age of 5 days in one of the above experiments was passed to three eggs 6 days old. On the 10th day after inocula-

Table II.

Activity tests on eggs inoculated with the FA strain.

Age of egg in days at time of in- oculation	Test made at time in days after in- oculation	Material tested	No. of mice inoculated	No. showing symptoms	No. immuno survivors
5	5	membrane	5	0	0
		CNS	5	0	0
		corpus	5	0	0
5	10	membrane	5	0	0
		CNS	5	5	—
		corpus	5	0	0
6	5	membrane	5	0	0
		CNS	5	0	0
		corpus	5	0	0
6	10	membrane	5	0	0
		CNS	5	5	—
		corpus	5	0	0
7	5	membrane	5	0	0
		CNS	5	0	0
		corpus	5	0	0
7	10	membrane	5	0	0
		CNS	5	2	0
		corpus	5	0	0
8	5	membrane	5	0	0
		CNS	5	0	0
		corpus	5	0	0
8	10	membrane	5	0	0
		CNS	5	0	0
		corpus	5	0	0

tion the eggs were opened, the CNS harvested, pooled, ground with sand and saline, and passed to a new set of three 6 days old eggs. At the same time a titration in mice was made. One more passage of the same kind was made and the last harvest inoculated into 15 eggs 10 and 11 days old. 10 days after inoculation the CNS was collected in the usual way and a pool of the material tested for activity in mice. The results of the titrations are summarized in table III.

The activity was thus maintained unchanged through four passages but the virus was still not infectious to older eggs.

Table III.

Activity tests on egg passages of the FA strain.  
Test made 10 days after inoculation.

Passage no.	Age of eggs at time of inoculation	Virus content of CNS (log MID per gm.)
2	6 days	4.15
3	6 "	4.80
4	6 "	4.15
5	10—11 "	no virus

Numerous sections of brain and spinal cord from infected embryos were examined microscopically. A few slides suggested a slight increase in the number of cells but nowhere any definite and characteristic lesions could be detected.

One more strain of mouse virus has been transferred to eggs. This strain, designated as UF I, of fecal origin, was of moderate virulence, the attack rate being about 80 p. c., the incubation time 10—15 days and the symptoms exclusively spinal. 6 days old eggs were inoculated and harvested, part after 5, the remainder after 10 days. No virus could be detected in the 5 days' harvest but two mice out of ten inoculated with CNS from the 10 days' harvest came down with typical symptoms on the 15th and 17th day.

Attempts were also made to transfer human poliomyelitis virus to eggs. For this purpose the L strain was employed, originally isolated by Levaditi in 1911 and since then maintained in monkey passages. Unfortunately these experiments had to be interrupted before any conclusive results were obtained. Even at the start of the experiments described above certain difficulties were encountered in procuring eggs of a satisfactory quality. In the winter 1941—42 when the attempt now mentioned was made, the scarcity of eggs was pronounced and obtainable hatching eggs of low quality, probably on account of vitamine deficiency of the chicken food. Most eggs, inoculated or not, died on the fifth to tenth day of incubation and it was practically impossible to carry through any passages even if as much as twenty eggs were used in each series. Under these circumstances the experiments were interrupted for the time being.

The experiments now described leave no doubt that the mouse poliomyelitis can be transferred to and propagated serially in the

developing chick, if indefinitely has not been settled as yet. Though this fact might be of a considerable theoretical interest, no practical application could be made. The immediate purpose of the experiments was to find a method for titration of the virus. As no characteristic lesions are produced, the diagnosis can be settled only indirectly through passage to mice, and transfer to eggs is therefore at present of no use either for titration or diagnostic purposes. As the virus concentration in the infected embryo remains very moderate, it offers no advantages as source of virus either.

### The incubation time method.

The main principles of this procedure have already been outlined by the present writer. As, however, accuracy in the measurement of biological activity is of the utmost importance for the solution of many problems of physico-chemical nature, a thorough examination of the method from this point of view was necessary. A survey of this kind must be based on a purely statistical reasoning, as individual variation is one of the most prominent characteristics of a biological material. In the choice of statistical methods the presentation by Bonnier and Tedin (15) of the theories of Fisher and others has mainly been followed.

In a routine titration a number of tests are compared with a standard. It is essential that all samples be derived from the same strain of virus and preferably of the same origin, the tests being different fractions or samples of the standard treated in different ways. The technique employed was in detail as follows.

From the standard a series of five tenfold dilutions was prepared, usually with log dilution 2, 3, 4, 5, and 6. From each test sample two dilutions were prepared: 0 and 2, 2 and 4, or 4 and 6, depending upon the range of activity to be expected. In diluting 1:10 1 ml was pipetted into 9 ml of diluting fluid, 1:100 0.25 ml into 25 ml. All pipet readings were made between marks and clean pipets used for each dilution. 0.05 M ( $\approx$  0.3 p. c.) sodium chloride solution was generally used for dilution. The inoculations were made with tuberculin syringes with glass pistons and needles gauge 20. The syringes were sterilized by boiling in water and before filling washed twice with the test sample in question. 30 days old mice served as test animals, the difference in age not exceeding 3 days. Uniformity of weight was usually not considered. From each dilution 0.02 ml amounts were inoculated intracerebrally into 10 mice.

With one assistant anesthetizing the animals it was possible to inoculate 400 mice per hour. The animals were kept by fives in glass jars, observed once or twice daily through at least three weeks, and their symptoms noted. When characteristic symptoms had appeared, the animals were sacrificed and the brains collected for purification purposes. After termination of the experiment all surviving animals were tested for immunity with a large intracerebral dose of the virulent FA strain.

In table IV the result of a typical titration experiment is tabulated. Each row represents a group of test animals inoculated with the same amount of the same dilution of a test material. The columns contain the numbers of animals, showing the first symptoms of infection on the day after inoculation which is indicated in the head of the column. The last one of these columns contains animals not becoming sick, the incubation period in these cases being indefinite.

Table IV.  
Result of a typical titration experiment.

Test material	Log dilution	Number inoculated	Number becoming sick after days											S(y)
			2	3	4	5	6	7	8	9	10	11	$\infty$	
12	2	10	1	6	3									3.25
	4	9			3	4	1	1						1.860
13	2	9		6	3									2.75
	4	10				9		1						1.943
14	2	10		2	7	1								2.617
	4	10				7			1	1			1	1.636
15	2	10		4	5	1								2.783
	4	10				6	2	1	1					1.801
16	2	10		6	3									2.75
	3	10			4	3	1	2						2.052
	4	10				6	1	3						1.795
	5	10					3	1	4	1			1	1.254
	6	10								1	1	1	7	0.302
17	2	10		2	7		1							2.583
	4	10			1	2		5	1				1	1.489

From a statistical point of view the experimental data form a problem of covariation and should be analysed accordingly. Independent  $x$ -variable is log dilution and dependent  $y$ -variable the re-

ciprocal of the incubation period. In the last column of the table the group totals of the  $y$ -values are listed.

To each test there corresponds a line of regression given by the equation

$$Y = \bar{y} + b(x - \bar{x}) \quad (7)$$

$\bar{y}$  and  $\bar{x}$  being the means of  $y$  and  $x$  and  $b$  the coefficient of regression, always negative when  $x$  denotes log dilution but positive when  $x$  denotes log concentration. This considered, equation (7) is identical with the incubation time formula (3) on page 35.

For each test, therefore, a coefficient of regression,  $b$ , can be calculated. The titer values obtained from the titration data are compatible only on the condition that the test groups show conformity with regard to regression of  $y$  on  $x$ . In a number of experiments the variation of  $b$  was studied from this point of view. The result, expressed as usual in terms of the probability of the differences observed being a chance variation, was as follows:

Probability (P)	No. of experiments
0.001—0.01	1
0.01—0.05	5
0.05—0.2	7
0.2—1.0	25
<hr/>	
Total 38	

In 32 out of 38 experiments the variation was probably due to chance. In 5 experiments a significance of the 1st order was observed, and in one a significance of the 2nd order. The result suggests largely a normal chance variation.

Provided the cause of variation in  $b$ , if not due to chance, be detected and eliminated, the titer values in an experiment like that in table IV can be calculated as follows. Let  $Y_0$  correspond to the dilution  $x = 0$ . According to equation (7) we have

$$Y_0 = \bar{y} - b\bar{x}. \quad (8)$$

$Y_0$  is a measure of the activity of the undiluted material. In order to obtain an expression of the usual form, i.e. in terms of log dilution we must divide through  $-b$ . Letting  $A$  be the activity, we get

$$\log A = \frac{\bar{y}}{-b} + \bar{x}. \quad (9)$$



Letting the index  $t$  denote a test sample and  $s$  the standard, we have

$$\log \frac{A_t}{A_s} = \frac{\bar{y}_t - \bar{y}_s}{-b} + (\bar{x}_t - \bar{x}_s). \quad (10)$$

The relation between  $A$  and the MID titer is expressed in the formula (4) on page 35. Considering that the constant  $c$  in this formula is approximately 1.0 and that the volume of the inoculum is 0.02 ml, the virus content might be estimated to approximately 5.4 MID per ml of the undiluted solution. This relationship will, however, be studied in detail later.

According to formula (9) the accuracy of the titer value is dependent upon the variation of  $x$ ,  $y$  and  $b$ . The former variation is principally connected with the accuracy of pipetting and can not be dealt with in this connection. The variation of  $y$  can be divided into two constituents, one of which refers to the regression of  $y$  on  $x$ , the other involves differences in the individual resistance to infection, errors of administration and distribution of the material inoculated etc.

### Variation of regression.

a) *Course of the line of regression.* In the analysis made above it was implied that the line of regression had a straight course. As already pointed out there is no easily conceivable theoretical basis for this assumption. As an entirely empirical observation its validity had to be statistically verified.

Suppose that the line of regression is curved. On that condition  $b$  in the formula (7) should be a variable, dependent upon  $y$ . This possibility can be analysed statistically by calculation of the regression of  $b$  on  $y$ . This was done in 26 different titration experiments. In the individual experiments the coefficient of regression ( $b$  on  $y$ ) was found to vary from  $-0.502$  to  $+0.728$  with a mean value of  $+0.035$ . With a  $P$  value of 1.0 it was not significantly different from 0. The coefficient of correlation was calculated to  $+0.105$ , this neither significantly different from 0, the  $P$  value being 0.2—1.0.

Furthermore in a series of experiments the deviation from a rectilinear regression was analysed in the usual way, and the probability of the deviation being due to chance was calculated. The result was as follows:

Probability (P)	No. of experiments
< 0.001	2
0.001—0.01	1
0.01—0.05	3
0.05—0.2	8
0.2—1.0	14
<hr/>	
Total 28	

Thus, in 22 out of 28 experiments the deviation observed was insignificant, in 3 significant of the 1st order, in 1 significant of the 2nd order and in 2 significant of the 3rd order. This distribution suggests a normal chance variation, except in two experiments, where the difference observed was probably due to technical inaccuracies.

There is, therefore, no evidence to indicate that the line of regression should deviate from a straight course in the interval used by titration. For extremely small or extremely great values of  $y$  one must expect a definite deviation. We will return later to this question.

b) *Technical errors* could certainly cause a variation of  $b$ . The amounts of solution inoculated are too small to be accurately measured with the syringes used, particularly if one considers the large series of animals to be inoculated in a single experiment.

*Experiment 4.* In a few experiments the influence on  $y$  and  $b$  of a variation in the volume of the inoculum was studied. Standard amounts of 0.01, 0.02, and 0.03 ml of the same virus solution, measured as accurately as possible, were injected intracerebrally to groups of 30 days old mice. The incubation periods were noted as usual. The results of three such experiments are summarized in table V.

The relative titers were calculated on the basis of a mean value of  $b$  derived from a very large series of experiments. In each case the virus content of the 0.01 ml dose was taken as standard, and the expected values of 0.02 and 0.03 ml calculated on this basis.

Table V shows considerable differences between found and expected. All those differences are positive. Furthermore their amount seems to be proportionate to  $y$ . This is illustrated in fig. 1, where the 0.02 ml values are fitted to the normal line of regression. The  $y$  values from the 0.03 and 0.01 ml groups were expected to fall on the two parallel dotted lines. The actual position of the points

Table V.

Influence on the incubation period of variation in the volume of the inoculum.

Experiment	Number inoculated	Volume inoculated	$\bar{y}$	Titer values	
				observed	expected
a	49	0.01	0.188	0.00	
	50	0.02	0.231	0.71	0.30
b	49	0.01	0.170	0.00	
	50	0.02	0.195	0.42	0.30
	50	0.03	0.222	0.87	0.48
c	50	0.01	0.292	0.00	
	48	0.02	0.351	0.98	0.30
	50	0.03	0.392	1.66	0.48

suggests that the lines of regression have a different slope, i.e. the value of  $b$  is variable, dependent upon the volume of the inoculum. The significance of this observation will be discussed elsewhere. In the present connection it is sufficient to remark that the variation in incubation period resulting from technical inaccuracy of administration is of a higher order of magnitude than the causative irregularities of dosage.

The best way to minimize the "personal" error in administration of the inoculum would probably be the use of spring steered stop syringes, automatically delivering a fixed amount of solution. On account of the existing circumstances it proved impossible to obtain instruments of that kind. The tuberculin syringes used instead were far from ideal for the purpose. They showed a marked tendency to leakage on the side of the piston, particularly when injection was applied against pressure, as is the case by intracerebral inoculations. The leakage was most pronounced when the piston was in topmost position and decreased when the piston was moved towards the bottom of the syringe. In order to standardize this error it was decided to fill the syringes only partially never exceeding a certain limit. Furthermore air bubbles must be removed. If not they would serve as air cushions and cause uncontrollable variation of dosage, when injection against pressure was applied. Finally, it was always

controlled that the total amount of material administered to one test group of animals was correct. This was done in order to make the total plus and minus errors as close to zero as possible.

One more source of error to be considered in this connection is the leakage from the site of inoculation through the needle puncture almost invariably occurring when the syringe is withdrawn. In a series of experiments on dead mice this leakage was found to be

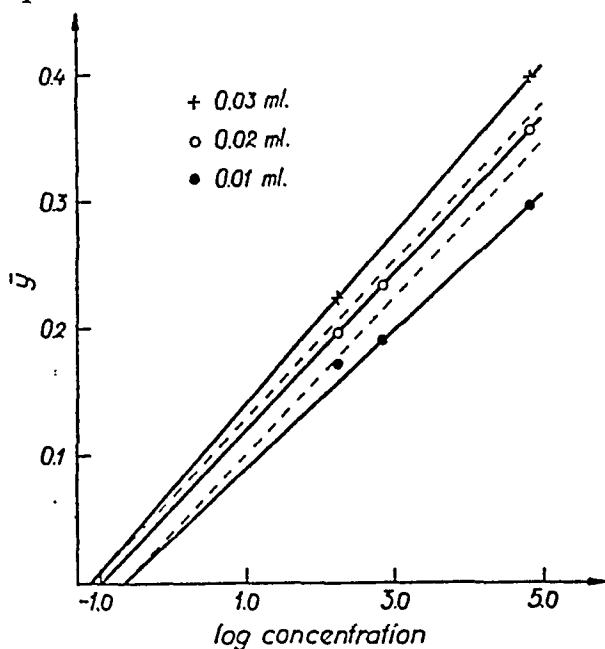


Figure 1. The influence of the volume of the inoculum on the length of the incubation period and the slope of the titration curve. Experiment 4.

astonishingly constant, amounting to about 0.002 ml. regardless of the quantity inoculated. In all calculations regarding absolute quantities, therefore, this correction should be made.

c) *The animal error.* In a series of experiments the individual resistance against infection and its dependence upon different factors were studied.

In all titration experiments each test group of ten animals comprised five males and five females. Calculations of the titer were done for both sexes separately. As the results regarding the relative resistance were invariably the same, it might be sufficient to quote one such experiment.



*Experiment 5.* Four unknown fractions from a centrifugation experiment were compared with the untreated standard. All technical details were in conformity with the general description on page 15. The result is summarized in table VI.

An analysis of the result showed with a probability  $P$  of more than 0.2 that the sexes behaved as random samples of the same statistical population. In particular the coefficients of regression,  $-0.05406$  and  $-0.05652$ , were identical with a probability of more than 0.2.

The influence of weight upon the resistance was analysed in the following experiments.

*Experiment 6.* A number of mice 31 to 34 days old were weighed in the morning before feeding and separated into groups according to weight, the mean weights of the groups being 9, 11, 13, and 17 g. Each mouse was inoculated intracerebrally with the standard amount of the same virus solution. The animals were observed as usual and the incubation periods noted.

Table VII.  
Weight and resistance.

Sex	Mean weight	Number inoculated	Number becoming sick after days					$\bar{y}$
			3	4	5	6	7	
a.								
♂	9	12	1	5	3	3	1	0.2236
	11	21	3	7	9	1		0.2314
	13	23	7	9	5	2		0.2573
	17	5	3	1	1			0.2900
♀	9	23	7	11	4	1		0.2630
	11	29	7	15	5	2		0.2557
	13	15	4	7	3	1		0.2567
b.								
♂	9	13	3	6	2	1	1	0.2469
	11	21	3	12	5	1		0.2460
	13	10	3	3	4			0.2550
♀	7	11	3	2	4	2		0.2394
	9	31	11	14	6			0.2699
	11	10	2	2	6			0.2367
	13	8	3	4	1			0.2750

In an identical experiment four groups of mice were inoculated. The mean weights were 7, 9, 11, and 13 g.

The results of these two experiments are summarized in tables VII a and b.

If these two experiments are analysed together one will find a regression of  $y$  on weight of  $+0.004$  and a correlation of  $+0.130$ , which with a probability of slightly more than 95 p. c. is different from zero. It is, thus, probable that big animals are less resistant than small animals of the same age. At first sight this fact might seem surprising. It must be kept in mind, though, that the big animals come from numerically small litters, the reduction in number of the young often being due to an abnormal mortality rate, indicating a low general resistance through inheritance. As the regression of  $y$  on weight, according to these experiments, was very low, it was not considered necessary to pay special attention to differences in weight among the test animals; the only precaution taken was to avoid letting any test group consist exclusively of unusually big or unusually small mice.

It has previously been pointed out that the age of the test animals has a very pronounced effect on the incubation period. This fact is clearly illustrated in the following experiments.

*Experiment 7.* Four groups of mice, 4, 6, 8, and 10 weeks old, were inoculated with the standard amount of the same virus solution. The animals were observed as usual and the incubation periods noted. The result is summarized in table VIII.

Table VIII.

Influence upon the incubation period of the age of the test animals.

Age of mice	$\bar{y}$	$\sigma$	
4 weeks	0.2897	0.05512	Regression of $\bar{y}$ on age $-0.012$ correlation $-0.385$
6 "	0.2527	0.05824	
8 "	0.2439	0.07427	Regression of $\sigma$ on age $+0.003$ correlation $+0.891$
10 "	0.2114	0.07197	

The experiment shows that  $y$  is dependent upon the age of the mice. The coefficient of correlation is  $-0.385$  and of a very high significance. Furthermore it can be seen from the table that the standard deviation ( $\sigma$ ) in a group of animals has a high positive

correlation to the age of the group. The coefficient of correlation  $+0.891$ , is, however, not quite significant.

*Experiment 8.* Infected mouse brains were ground with sand and saline to a 10 p. c. suspension and centrifuged. From the supernatant a series of 5 tenfold dilutions was prepared. From each dilution two groups of test animals were inoculated intracerebrally with the standard amount. One group comprised mice, 43 days old, the other group consisted of animals the age of which varied from 54 to 92 days. The animals were observed as usual and the incubation periods noted. The result of the experiment is summarized in table IX.

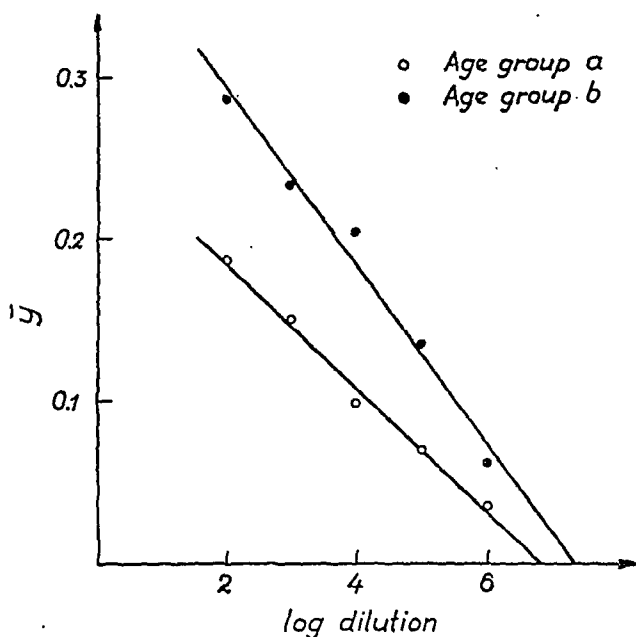


Figure 2. Influence of the age of the test animals on the incubation time curve. Experiment 8.

The experiment showed that not only  $y$  but also  $b$  is dependent upon the age of the test animals. The coefficients of regression were  $-0.03843$  and  $-0.05559$ . The probability of this difference being due to chance variation is less than 5 p. c. The experiment is illustrated graphically in fig. 2.

The last experiment demonstrates the limited value of an absolute titer, in terms of MID or otherwise. The titer of a given virus solution is dependent not only upon the virus content but also upon the resistance of the test animals used for titration. Only a comparison between two titer values is of any significance, either as measurement



sieurs couches cellulaires. Ces images ressemblent très fortement à celles décrites par Merckel.

Les grandes artères, situées en profondeur, présentent un endothèle aplati avec une couche sousjacent de tissu conjonctif riche en cellules qui sont entourées par des membranes élastiques. Ces cellules ont une direction différente des cellules des couches plus externes vis-à-vis desquelles elles semblent perpendiculaires ou obliques.

Dans la *sousséreuse* où l'on trouve assez bien ou beaucoup de tissu conjonctif de néoformation, les capillaires présentent un endothèle turgescent. Il en est de même pour les petites artérioles qui montrent une fine couche sousendothéliale, les noyaux de la couche musculaire sont également turgescents. Le tissu conjonctif entre les cellules musculaires a encore fortement augmenté dans les grands vaisseaux. Ces cellules musculaires encerclées ont des noyaux sphéroïdes, à disposition irrégulière par l'accroissement du tissu conjonctif collagène; leur cytoplasme est clair. La coloration de l'élastine démontre que ces cellules sont entourées de fibres élastiques bien que quelques unes ne présentent de l'élastine que du côté central.

*Conclusion:* en cas d'ulcère du bulbe avec peu de néoformation de tissu conjonctif;

1) les capillaires présentent un endothèle turgescent; d'ailleurs peu accusé s'il y a peu de tissu conjonctif de néoformation mais bien de la gastrite.

2) les artères moyennes présentent une couche conjonctive sousendothéliale avec des cellules désordonnées, délimitées par de l'élastine du côté central; d'autres vaisseaux artériels montrent plusieurs couches de cellules et de noyaux désordonnés entourées d'élastine. (Photo 5.)

3) les grandes artères présentent une plus forte néoformation de tissu conjonctif, une forte formation de couches élastiques stratifiées et une transformation progressive *centripète* des couches cellulaires pseudo-proliférantes.

### *B. Ulcère du ventricule.*

1) *En dehors de l'ulcère, où l'on ne rencontre pas de tissu conjonctif de néoformation, les artères ont un aspect normal.* (Photos 1 et 2.)

Dès qu'on se déplace vers la zone périulcéreuse macroscopiquement caractérisée, au point de vue artériel, par un rétrécissement

Table IX.

Influence of the age of the experimental animals upon the result of titrations.

Age group *a* 54—92 days old, age group *b* 43 days old.

Age group	<i>x</i>	No. inoculated	Number becoming sick after days																				$\bar{y}$	<i>b</i>	log <i>A</i>		
			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	24				$\infty$	
<i>a.</i>	2					3	2	2	1			1											2				
	3		2	6		6	2	4	1				1					1						8			
	4				1	4		5	1		1		2					1					14				
	5						4	4	2	3	1				1								20				
	6							2	2	2	1	1			1	2	1		1								
																										0.0972 -0.03843 6.79	
<i>b</i>	2	1	2	3	1																						
	3		3	3	2	2	1	1																			
	4		2	1		2		3																			
	5					1	1		2																		
	6					1	1	1	1																		
																											0.1849 -0.05559 7.28

incubation period for each animal an average must be instituted. The arithmetic mean for day 2 should be 1.75. The corresponding value of  $y$  can be calculated by integration of the function  $y = 1/t$  in the interval 1.25—2.25 and is found to be 0.5878. There is a distinct difference between this figure and the value accepted for calculation of the titer. The difference, amounting to 17.5 p. c. for day 2, rapidly decreases with increasing incubation period, being only 2.65 p. c. for day 10.

The two circumstances now mentioned both act in the same general direction, towards a lowering of the value of  $y$ . The difficulty in determining the exact incubation period mainly affects the small  $y$  values, the approximated calculation of the average principally the large ones. It is thus possible that the total effect might be a parallel displacement of the titration curve, although it is more probable that the first effect in question does not counterbalance the second. It is therefore reasonable to assume that the relationship between  $y$  and log dilution in reality is not linear but represents a curve with the convexity towards the  $x$  axis.

As already shown the formula (7) holds true in the interval ordinarily used by titration, with the approximated calculation of incubation periods as originally accepted. From a practical point of view there has been no reason, therefore, to substitute for this procedure a more complicated one even if this might be more correct. From a theoretical point of view, on the other hand, the facts now mentioned are most important and their significance will be discussed in another connection.

In the course of two years a considerable number of titrations were carried out, in which the principles outlined above concerning the uniformity of the test animals were strictly followed. In a series of 34 such experiments comprising more than 4000 test animals the variation in the value of  $b$  was studied. The figures obtained suggested a seasonal fluctuation of the general level of resistance. A statistical analysis showed, however, that the differences were within the limits of a chance variation. It might be possible to present conclusive evidence in this matter in the future, when a larger material has been collected. As conditions now were, it was considered most adequate to compute a mean value of  $b$  to be applied to all calculations of titer values. This was found to be  $b = -0.05473 \pm 0.00077$ .

### Variation of $y$ .

The variation of  $y$  is measured by the mean square residual ( $\sigma^2$ ), obtained after elimination of the part of the variation that refers to the regression of  $y$  on  $x$ . It is obvious that all the sources of error that were reviewed on the preceding pages contribute to this variation. There exists, however, one more cause of variation not yet considered. By random sampling from a suspension of particles in a fluid, the number of particles per sample will show a frequency distribution according to the law of Poisson. This distribution error is a quality inherent in the particulate nature of the virus, and cannot be influenced by any technical device. On certain theoretical conditions to be discussed in the following chapter the amount of this variation can be calculated. For  $\bar{y} = b$  it amounts to about 0.0010 and decreases rapidly with growing  $\bar{y}$ .

The same series of experiments, from which the average value of  $b$  was computed, was used for calculation of  $\sigma$ . A mean value of  $\sigma^2 = 0.003427$  was obtained. As the above mentioned distribution error contributes but an insignificant part, the variation must be caused by technical inaccuracies. In order to enhance the accuracy of titration there is, therefore, more to be gained by technical improvements than by increasing the number of test animals.

From the value of  $b$  and  $\sigma$  the standard error of the titer can be calculated. For 5 groups of 10 mice each one gets  $\varepsilon(\log A_t) = \pm 0.158$ , and for 2 groups of 10 animals  $\varepsilon(\log A_t) = 0.245$ , why the difference between test and standard in a routine titration has a standard error  $\varepsilon\left(\frac{\log A_t}{\log A_s}\right) = 0.292$ .

In the following experiment the actual accuracy of the method was tested.

*Experiment 9.* Infected mouse brains were ground with sand and saline to make a 10 p. c. suspension, and centrifuged in an angle centrifuge to remove sand and coarse material. The supernatant = solution a. Part of this was diluted with 1/2 volume of saline = solution b. Of each solution a series of tenfold dilutions was prepared and of each dilution the standard amount inoculated intracerebrally to 10 mice of standard age. The animals were observed and readings made as usual. The result is summarized in table X.

According to the above calculations the standard error of each titer value would be 0.158, and that of the difference consequently 0.223. The actual difference in concentration between a and b



amounts to  $\log A_a - \log A_b = 0.176$ . The difference observed in the experiment was 0.258. The error is therefore 0.082, i.e. well within the limits of the standard error.

\*   \*   \*

Finally it must be pointed out that the incubation time titration method has only a limited field of application. Most strains of mouse poliomyelitis virus, including all intestinal strains, give symptoms exclusively from the spinal cord. When inoculated intracerebrally, they show a practically constant incubation period, regardless of the amount of virus injected. So far only two strains have been isolated, regularly displaying encephalitogenic properties. These strains, designated as GD VII and FA, were both picked up accidentally in passage series of yellow fever virus, and have been described by Theiler and Gard (150). It is only on these strains that the incubation time method of titration can be applied and only after intracerebral inoculation. If the animals are infected by the intranasal or the intraperitoneal route the incubation period will have a constant length regardless of the amount of virus inoculated.

### The 50 p. c. endpoint method.

In chapter IV page 66 a large scale experiment on the determination of the 50 p. c. point is reported. In this experiment 15 groups of 50 mice each in the range between 0 and 100 p. c. takes were observed. The standard deviation of percentage of takes from the best fitting distribution curve was calculated to 5.94. When groups of 10 mice instead of 50 are used, the standard deviation would amount to 13.28. If the titration is done with tenfold dilutions an average of 2.7 mouse groups will give a result in the range between 0 and 100 p. c. takes, meaning that 2.7 groups can be used for calculation of the titer value. The standard error of the computed 50 p. c. point will, therefore, be 8.08. The slope of the titration curve in the interval in question, i.e. the increase in percentage of takes per unit of log dilution can be estimated to approximately 40. The standard error of the titer value, expressed in terms of log dilution will, therefore, be  $\frac{8.08}{40} = 0.20$ .

For titration of an unknown sample according to the 50 p. c. endpoint method usually at least 5 groups of animals must be inoculated in order to ascertain that the favourable interval is covered. For a simultaneous titration of, say, seven different samples 350 mice had to be used. The difference between two titer values could then be estimated with an accuracy of 0.285. With the incubation time method only 170 mice had to be used and the standard error would according to the deductions on page 55 be 0.292, i.e. practically the same accuracy with less than half the number of test animals. For simultaneous titration of a large number of test samples the incubation time method is, therefore, to be preferred.

In a number of suitable experiments the 50 p. c. endpoint titer was calculated together with the log  $A$  from the incubation time. In table XI these titer values are compared.

Table XI.

Comparison between 50 p. c. endpoint and incubation time titer.

Incubation time titer	50 p. c. endpoint titer	Difference
6.97	5.75	1.22
4.00	2.88	1.12
8.14	6.25	1.89
7.69	6.58	1.11
7.41	6.25	1.16
6.48	4.58	1.90
7.46	5.75	1.71
6.86	5.25	1.61
7.42	6.00	1.42
7.16	5.67	1.49
6.65	5.67	0.98
5.28	3.77	1.51
5.68	4.11	1.57
6.56	6.38	0.18
6.58	5.88	0.70
6.42	5.30	1.12
7.38	6.39	0.99
7.16	5.86	1.30
7.63	6.36	1.27
Average		1.277

centrale à noyaux ronds ou sphéroïdes, désordonnés, plus ou moins enrobés dans le tissu conjonctif; une couche plus périphérique de cellules musculaires circulaires à noyaux fusiformes; le tout est entouré par l'adventice.

Les artères plus grandes présentent plus de tissu conjonctif de néoformation; une élastique interne n'existe pas mais la coloration à la Weigert-résorcine montre un réseau de fibres élastiques dans le tissu conjonctif. (Photo 6.)

*Il semble que le tissu conjonctif entre les cellules musculaires de la média augmente de sorte que les cellules musculaires typiques de la média changent progressivement de direction; leurs noyaux allongés prennent une direction de plus en plus oblique et perpendiculaire vis-à-vis de l'axe de l'artère, ils s'arrondissent.* Les couches cellulaires périphériques sont nettement fibrillaires, tandis que les cellules enrobées dans le tissu conjonctif néoformé perdent rapidement leur caractère fibrillaire, ils présentent un cytoplasme clair. *On peut suivre tous les stades de transformation des cellules musculaires typiques en cellules polymorphes de la couche proliférante bien qu'il n'y ait qu'une seule couche de transition des cellules de la média.* Le tissu conjonctif de la média authentique (c.à.d. à morphologie inchangée) est en continuité intime avec le tissu conjonctif de la couche proliférante.

La disposition capricieuse des cellules et des noyaux est due à la prolifération inégale du tissu conjonctif intercellulaire. Les fibres élastiques forment un réticulum dans le tissu conjonctif proliférant; ce réticulum est incomplet et certaines cellules apparaissent comme uniquement entourées d'élastine à leur face centrale. *Dans la même artère on peut souvent retrouver tous les stades d'encerclement des cellules de la média par le tissu élastique, processus d'intégration dans la soi-disant intima.* (Photo 4.)

Dans les artères de calibre moyen, la couche proliférante est parsemée de fibres élastiques, la couche la plus périphérique étant la plus épaisse: elle représente »l'élastique interne«. (Photo 6.)

c) *à proximité de l'ulcère:* on observe un accroissement remarquable de tissu conjonctif. Dans les petites artères de toutes les couches (sous-muqueuse, musculaire et sous-séreuse) il apparaît une forte néoformation de tissu conjonctif intercellulaire dans la média, de sorte que certaines cellules de la média semblent incrustées.

Les artérioles présentent un accroissement progressif de la substance intercellulaire conjonctive collagène d'autant plus pronon-



## On the theory of titration with reference to certain physico-chemical properties of the virus.

When random samples of a constant quantity are picked from a suspension of particles in a fluid medium the number of particles per sample will show a certain variation around the average. If the distribution of the particles in the medium is due exclusively to chance, i.e. if no attraction or repulsion between particles influences the equilibrium, the frequency series of Poisson can be applied to the problem of calculating the expected number of particles per sample. Let  $x$  be the average number per sample. Then in a sufficiently large series of samples the fraction

$$\begin{array}{llll} e^{-x} & \text{will contain no particles} & & \\ x e^{-x} & \text{»} & \text{»} & 1 \text{ particle} \\ \frac{x^2}{2!} e^{-x} & \text{»} & \text{»} & 2 \text{ particles} \\ \frac{x^n}{n!} e^{-x} & \text{»} & \text{»} & n \text{ »} \end{array}$$

The probability of a sample containing at least one particle will, therefore, be

$$P = 1 - e^{-x} \quad (11)$$

Let the particles be bacteria and the samples used as inocula in a series of cultures. The expression (11) will then give the expected fraction of positive results.

In the case of a virus titration conditions are partially different. Poisson's law must of course be applicable to the distribution of particles in the suspension, but the percentage of takes cannot be calculated as in (11). When the sample is injected into a test animal, the fluid is distributed over a certain amount of tissue. In this

tissue there are a limited number of susceptible cells, serving as entry points. Thus usually only a fraction of the virus particles inoculated have a chance to infect the host. Let the probability of hit be  $p$ . If the sample contains  $n$  particles, the probability of no hit will be  $(1 - p)^n$ . The total probability of takes is obtained by addition of the series

$$P = [1 - (1 - p)] x e^{-x} + [1 - (1 - p)^2] \frac{x^2}{2!} e^{-x} + \dots$$

$$+ [1 - (1 - p)^n] \frac{x^n}{n!} e^{-x} + \dots$$

$$P = 1 - e^{-px} \quad (12)$$

If the functions (11) and (12) are reproduced graphically with  $P$  on the ordinate and  $\log x$  on the abscissa, one will find that the curves have exactly the same shape, but that (12) is displaced in comparison with (11) with the amount  $-\log p$ . From the shape of the distribution curve, therefore, nothing can be learnt about the real values of  $p$  and  $x$ . Only their product can be calculated.

The virus entity one MID does not tally with a fixed number of virus particles. On the contrary it is variable with  $p$ , which in its turn is an expression for the equilibrium between host resistance and pathogenicity of the given strain of virus. Letting  $v$  be the virulence of the strain and  $r$  the resistance of the host,  $p$  can be given the form  $p = \frac{v}{r}$ . Therefore,  $p$  is a measure of the virulence as well as of the resistance. As most of the following deductions are concerned with the latter aspect,  $p$  will be called the resistance factor of the probability function.

In the case of vaccinia virus a whole titration can be done in one animal, a procedure guaranteeing the best possible constancy of  $p$ . In most instances, however, a comparatively large number of animals must be included. One has, therefore, to expect a certain variation in  $p$ , so that the shape of the distribution curve will deviate from that of function (12). The actual curve can be regarded as an average of a number of normal curves with different  $p$  values, and its shape will be dependent upon the type of variation of resistance. At any rate it must have a flatter course than the normal distribution curve.

As already mentioned Parker (112) tested the validity of function (12) on vaccinia virus. His aim was to ascertain whether or not one

single elementary body was sufficient to cause infection. Consequently he studied the distribution curve in the range between 0 and 100 p. c. takes, comparing the experimental data with function (12) and with a series of functions easily derived from this one, expressing the probability of takes if 2, 3, etc., virus particles form a minimal infective unit. These functions are

$$P_2 = 1 - (1 + px) e^{-px} \quad (12a)$$

$$P_3 = 1 - \left( 1 + px + \frac{(px)^2}{2!} \right) e^{-px} \quad (12b)$$

etc.

Each one of these curves has a steeper slope than the normal function, and steeper the higher the order. Parker found good agreement with (12), but none with the curves of a higher order. Haldane (57), however, pointed out certain inadequacies in Parker's calculations. When these were corrected the result indicated a systematic deviation from the normal curve. Bryan and Beard (19) suggested that this deviation was caused by variation in resistance among the rabbits, and showed that Parker's figures agree with function (12), if a normal distribution of animal resistance was assumed.

Parker, Bronson and Green (113) were able to confirm this in a later series of experiments with different strains of virus. In one of these strains, the culture virus C V I with a very low intracutaneous pathogenicity, however, a marked disparity between experiment and theory was observed. The data were best explained by the assumption that as many as 10 elementary bodies were necessary to cause infection. The authors suggest the explanation that one single focus of infection is insufficient to cause a visible reaction. Not until the density of foci exceeds a certain critical level, the reaction becomes recognizable.

If this interpretation is correct, which seems very plausible, it is, however, questionable whether the formulas (12a) etc. should be applied. A comparison between the egg membrane and the intracutaneous titers of the strain C V I indicates that  $p$  in this case is of the order of magnitude  $10^{-5}$ . When in a test 0.1 ml is inoculated intracutaneously, the inoculum, therefore, can come in contact with but a very limited number of susceptible cells. It is evident that, with a certain number of hits, the chances that more than one hit

occurs in the same cell are greater, the smaller the number of cells in which the hits are to be distributed. But according to Parker it is the number of foci not the total number of hits that count.

The amount of tissue infiltrated by the inoculum will be called the hit area. Let the number of susceptible cells within this area be  $a$ . In the case of  $n$  hits the probability, that all occur in the same cell, is  $\frac{1}{a^{(n-1)}}$ . The probability of 1, 2, 3, etc. hits is  $pxe^{-px}$ ,  $\frac{(px)^2}{2!}e^{-px}$ ,  $\frac{(px)^3}{3!}e^{-px}$ , etc. Thus, the total probability of one focus is

$$P_{(1)} = pxe^{-px} + \frac{1}{a} \frac{(px)^2}{2!} e^{-px} + \frac{1}{a^2} \frac{(px)^3}{3!} e^{-px} + \dots$$

$$P_{(1)} = a(e^{px/a} - 1)e^{-px} \quad (13)$$

On the condition, that 2 foci are necessary to give a visible reaction, the probability of takes will be

$$P_2 = 1 - [1 + a(e^{px/a} - 1)]e^{-px} \quad (14)$$

In an analogous way the functions of higher order can be derived.

When the formulas of type (12a) etc. are compared with those of type (14), one will find that the former represent the limit values of the latter for  $a = \infty$ . An appreciable deviation does not occur until  $a$  has the value of or below 10. The principal difference is a displacement towards greater  $px$  values, whereas the slope of the curve is practically unchanged. When only calculation of the slope of the titration curve is concerned, the approximated formulas (12a) etc. can be applied, so Parker's figures must be regarded as satisfactory.

In the above reasoning it was assumed that a single elementary body was capable of inciting infection, although the intensity of the process was so low as to make a single focus imperceptible. It might be, however, that the low virulence of the C V I strain is due to a deficiency in some activator necessary for establishment of intracutaneous infection (for instance the soluble polysaccharide antigen). In this case two alternatives are possible.

a. The activator is inherent in the virus particle. Then more than one particle must enter into the same cell to give the amount of activator necessary. Only the case of two particles forming a minimal infective unit will be considered. The symbols are as before. The probability, that  $n$  hits all occur in different cells is

$$\frac{a!}{a^n(a-n)!}$$

and, therefore, the probability of at least one double hit  $1 - \frac{a!}{a^n(a-n)!}$ . If  $n$  is greater than  $a$  it is self-evident that double hits must occur. The total probability of takes is then

$$P = \left[ 1 - \frac{a!}{a^2(a-2)!} \right] \frac{(px)^2}{2!} e^{-px} + \dots + \left[ 1 - \frac{1}{a^a} \right] \frac{(px)^a}{a!} e^{-px} + \frac{(px)^{(a+1)}}{(a+1)!} e^{-px} + \dots$$

By separating the members of the bracket one gets

$$P = S \sum_{n=2}^{\infty} \left[ \frac{(px)^n}{n!} e^{-px} \right] - S \sum_{n=2}^n \left[ \binom{a}{n} \left( \frac{px}{a} \right)^n e^{-px} \right]$$

and

$$P = 1 - \left( 1 + \frac{px}{a} \right)^a e^{-px}. \quad (15)$$

b. The activator is mainly present in the fluid medium and does not combine with the virus until *in loco*. Only the simple case that one activator molecule together with one virus particle forms the minimal infective unit will be considered. The total hit area is  $\frac{a}{p}$ . If one cell is hit by a virus particle, the probability that an activator molecule will hit the same cell is  $\frac{p}{a}$ , and the probability of no hit consequently  $1 - \frac{p}{a}$ . The probability that of  $n$  activator molecules none will hit the cell is  $\left( 1 - \frac{p}{a} \right)^n$ . Suppose the solution contains an average of  $c$  activator molecules per virus particle. The probability of takes in the case of one hit by a virus particle will then be

$$P_{(1)} = S \left\{ \left[ 1 - \left( 1 - \frac{p}{a} \right)^n \right] \frac{(cx)^n}{n!} e^{-cx} \right\} = 1 - e^{-cpx/a} \quad (16 a)$$

In the same way the probability of takes in the cases of 2, 3, etc. hits by virus particles can be calculated

$$P_{(2)} = 1 - e^{-2cpx/a}$$

$$P_{(3)} = 1 - e^{-3cpx/a}$$

etc.

To obtain the total probability of takes one has to multiply each expression (16 a) etc. with the corresponding probability of 1, 2, 3, etc. foci<sup>1</sup> and add the

<sup>1</sup> These values can be calculated in analogy with function (13), the probability of  $n$  hits being distributed over  $m$  foci being

$$P_{(m)}^{(n)} = \binom{a-1}{m-1} \frac{m^{n-1} - \binom{m-1}{1} \cdot (m-1)^{n-1} + \binom{m-1}{2} \cdot (m-2)^{n-1} \dots + (-1)^n}{a^{n-1}}$$

products. This leads to a rather complicated formula. In order to get an idea as to the form of this formula we will instead of the probability functions mentioned use the approximated expressions of the form  $\frac{(px)^n}{n!} e^{-px}$ , remembering that the function ensuing is strictly valid only for small values of  $px$  and large values of  $a$ .

$$P = S \left[ (1 - e^{-ncpx/a}) \frac{(px)^n}{n!} e^{-px} \right] = 1 - e^{-px(1 - e^{-cpz/a})} \quad (16)$$

The principal difference between the functions (14), (15), and (16) lies in their dependence on the arbitrary constant,  $a$  resp.  $c/a$ . (14) was practically independent of the value of  $a$ ; only a slight displacement towards greater  $px$  values occurred with decreasing  $a$ . (15) and (16) on the other hand are largely influenced by variations of the constant and the displacement has the opposite direction, i.e. towards greater  $px$  values with increasing  $a$ .

It is certainly possible to change the value of  $a$  within certain limits by using inocula of different volume. A large inoculum will infiltrate a larger amount of tissue, and the number of susceptible cells in the hit area will be greater than when a small volume is inoculated. It would, therefore, be theoretically possible to decide whether a function of type (14) or of the other type were the most probable. An experiment to that effect has, in fact, been reported by Sprunt (140, 141). He titrated the virus, using different standard volumes at inoculation, and found that the activity calculated was higher, the smaller the volume of the inoculum. His conclusion was that the effect was dependent upon the concentration of the virus rather than the actual number of virus particles inoculated. This observation is decidedly in favour of one of the functions (15) or (16). There is, however, one source of error in an experiment of this kind, to which special attention must be paid, before definite conclusions could be drawn. Intact and normally functioning cells seem to be a necessary condition for the multiplication of the virus. When large volumes are inoculated intracutaneously, distortions of the tissue might easily occur, injuring a number of cells so as to make them unsuitable as substrate for the virus. Sprunt's experiment ought to be controlled with due attention to this possibility.

## Observations on mouse poliomyelitis.

With the FA strain of mouse poliomyelitis virus an experiment of the same kind as Parker's was carried out.

*Experiment 10 a and b.* In a preliminary titration the 50 p. c. endpoint of an ether-treated extract of infected mouse brain was determined. In accordance with the titer value thus obtained a series of 8 twofold dilutions was prepared, calculated to cover the entire range between 0 and 100 p. c. takes. From each dilution standard amounts of 0.02 ml were inoculated into groups of 50 mice of standard age. The animals were observed during 4 weeks and the

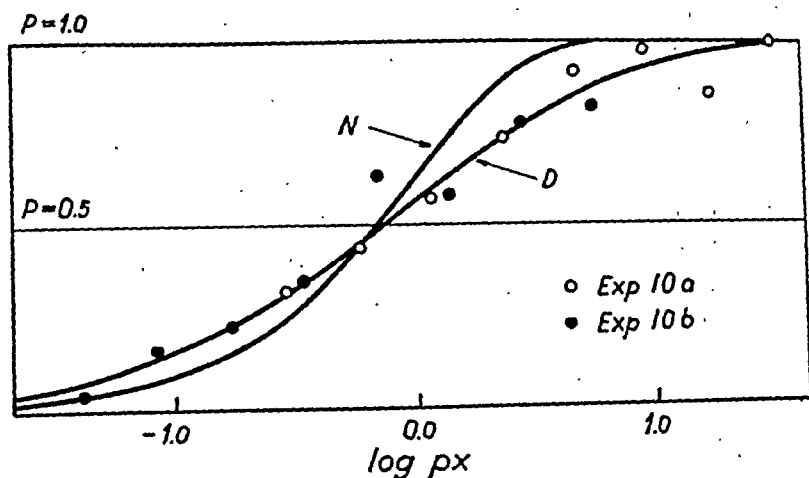


Figure 3. Probability of takes. Curve N = normal distribution. Curve D = distribution in test animals of varying resistance against infection. Experiment 10.

incubation periods noted as usual. The concentrations of virus used turned out to be slightly too high, so an identical experiment was set up with concentrations being 0.15 of those in the first series. In the interval between the experiments the virus solution was stored in the refrigerator, under which conditions the activity remains unchanged for several weeks. The results of the two experiments were very conform, and they will, therefore, be analysed as one single experiment. The result is summarized in table XII.

The 50 p. c. point was calculated according to Reed and Muench, and was found to correspond to the concentration  $10^{-6.778}$ . In fig. 3 the distribution function (12) is reproduced graphically (curve N) and the experimental data plotted so as to let the 50 p. c. point coincide with that of the curve. It is obvious at first sight, that there is a systematical deviation from the expected values. This is confirmed by a statistical analysis. One obtains a  $\chi^2$  total of 55.56 corresponding to a probability of identity far less than 0.001. The actual titra-

Table XII.

log dilution	No. inoculated	No. becoming sick after days																				Per cent positive	Per cent single mol.
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	∞			
Experiment 10 a.																							
5.000	49	10	7	22	7	2			1												8	100.0	0.0
5.301	48	1	4	14	11	3	4	2	1												1	85.4	0.0
5.602	46		1	10	12	9	5	5	2	1											4	97.8	0.0
5.903	48		2	13	6	7	6	4	3	3											13	91.7	0.0
6.204	49	1		2	8	10	6	7	1		1										22	73.5	0.0
6.505	50			1	7	7	5	4	3		1										27	56.0	0.0
6.806	48				3	7	1	3	4			1	1	1							32	43.7	4.8
7.107	47					2	5	2		3					1	1					32	31.9	20.0
Experiment 10 b.																							
5.824	50			6	14	11	4	4			1										9	82.0	2.4
6.125	49			2	11	18	3	2			1								1		11	77.6	2.6
6.426	50				12	10	2	2	1										1	1	21	58.0	6.9
6.727	49			1	5	13	1	4	2	2					1	2					18	63.3	9.7
7.028	49				2	3	2	4	1	1				1	1	1	2				32	34.7	23.5
7.329	49					1	5	1						1							38	22.4	36.4
7.630	49								2	1	1					1	1	1			41	16.3	37.5
7.931	50								1									1			48	4.0	50.0
Total		12	14	71	98	94	49	45	21	11	5	1	1	1	6	5	5	5	1				



tion curve has a course that is decidedly flatter than that of the normal distribution curve. It is, therefore, obvious that the agreement with the different types of curves hitherto discussed must be even less satisfactory, all of these having a steeper slope than the function (12). Accordingly there is no reason to suppose that the minimal infective unit contains more than one virus particle.

The percentage of takes as calculated according to the formula (12) corresponds to the number of particles per inoculum. The result of experiment 10 might, therefore, indicate that the relative number of particles decreased with increasing concentration, i.e. that the unit particles were associated to larger aggregates. On dilution a process in the opposite direction would occur. By dissociation of virus aggregates two principally different types can be anticipated. (a) Through combination with inhibiting or neutralizing substances inactive aggregates are formed, on dilution liberating active virus particles. (b) There is a tendency to formation of active aggregates or to dissociation into single virus molecules towards an equilibrium dependent upon the concentration of virus.

Both types of reversible dissociation have been observed. The most extensive study in this respect has been made on plant viruses. Mulvania (105) found in 1926 that a partial neutralization of the infectivity of tobacco mosaic virus containing plant juice could be obtained by addition of normal rabbit serum. The infectivity could be restored to a large extent simply through dilution, i.e. a partially neutralized mixture shows a deviation from the normal formula for the distribution of takes. The same phenomenon and even more strikingly is observed at dilution of a mixture of virus and specific antiserum.

Bald (4) found, that crude infective plant juice gave a dilution curve definitely different from the expected one, whereas purified virus showed a high goodness of fit. He stated, however, later (5) that certain pure preparations exhibit the same phenomenon and suggested that it was the result of a dissociation of aggregates. That aggregation can occur has been shown through filtration and stream double refraction measurements by Bawden and Pirie (8) and by Loring, Lauffer and Stanley (100) and the latter authors present evidence to show that this aggregation is reversible by dilution under certain conditions.

From animal virology it is known that the infectivity of partially neutralized viruses can be restored to a certain extent through dilu-

tion, at least in fresh mixtures of virus and serum. The second type of dissociation, however, has not been observed.

Before a discussion of the different possibilities in our case the following remark about the material in table XII should be made. In an experiment of this kind one must expect that a number of animals were infected by a single virus particle, and that furthermore groups of mice could be distinguished infected by 2, 3, etc. particles. According to the formula (7) on page 43 each such group of animals would be characterized by a certain average incubation period. The variation in resistance would, however, scatter the individual values about the average and probably cause an overlapping of successive groups, making them partly indistinguishable. Let  $t_x$  signify the incubation period in the case that  $x$  particles incited the infection. By inversion of the formula (7) we get

$$\frac{1}{t_{x+1}} - \frac{1}{t_x} = -b \log \frac{x+1}{x}$$

It is easily shown that the difference in incubation period between two consecutive groups will decrease with increasing  $x$ . If therefore, any group at all can be distinguished, it should be the one corresponding to infection with a single virus particle, i.e. with the maximum incubation period. From the totals in the last row of table XII it is evident, that one such group is clearly distinguishable in the range from 16 to 21 days, comprising 23 animals. The average  $\bar{y}$  was calculated to 0.0545, corresponding to an incubation period of 18.35 days. On the basis of this value the incubation period of the 2 particle group was calculated to 14 days in reasonable agreement with the experimental data.

On the assumption that this interpretation of the experiment is correct, the last column was added to table XII, where the single molecule infections are recorded in percentage of the total number of takes in the corresponding group of animals. After this preliminary remark the discussion of the distribution curve will proceed.

*Alternative A.* The union of virus and neutralizing substance has been studied extensively by Burnet (22). The percentage law (Elford and Andrewes), saying that the proportion of non-neutralized virus particles is independent of the concentration of virus, was explained on the assumption that Langmuir's adsorption isotherm

was applicable to the equilibrium between virus and antibody. It can be formulated

$$kc = \frac{100 - P}{P}$$

$k$  being a constant,  $c$  the concentration of antibody, and  $P$  the percentage of «surviving» virus particles.<sup>1</sup> Let  $x$  be the total concentration of virus,  $x_0$  the observed concentration, i.e. the number of active particles per unit volume. In our case  $\frac{c}{x}$  will be constant. Burnet's formula can, therefore, be given the form

$$x_0 = \frac{x}{1 + kx}. \quad (17)$$

With increasing  $x$ ,  $x_0$  will approach an upper limit  $\lim x_0 = \frac{1}{k}$ , i.e. regardless of the concentration of the material the number of active particles per unit volume will never exceed this value. From the probability function (12) it can be calculated that with  $px_0 = 5$  the probability of takes is 99.32 p. c., i.e. out of 1000 animals inoculated an average of 7 would remain symptomfree. Among several thousands of mice, inoculated with concentrated solutions of FA virus, no skips have been observed. It is, therefore, evident that in the present case the following inequality must be valid

$$\lim px_0 = \frac{p}{k} > 5$$

or

$$k < 0.2 \ p$$

$p$  can be estimated to about 0.01 and  $k$ , therefore, must have a value below 0.002. In that case, however, the distribution curve obtained through substitution of  $x_0$  for  $x$  in function (12) will not deviate with an appreciable amount from the normal curve. The percentage law is, therefore, not applicable to the present case.

If on the other hand the law of mass action is applied to the formation of inactive aggregates of virus and inhibitor, one might reason

<sup>1</sup> According to Merrill (103 a) the inactivation is only apparent, the reduction in infectivity instead being due to agglutination of virus particles. In the present connection the interpretation of the phenomenon is of secondary interest as long as the number of active particles, aggregated or not, can be calculated by means of the given formula.

as follows. Suppose that the inactive complex contains  $m$  virus particles and  $n$  inhibitor molecules. Let  $x$  be the total number of virus particles per unit volume and  $x_0$  the number of free particles. The number of inactive complexes will then be  $\frac{x - x_0}{m}$ . Let further  $a$  be the concentration of free inhibitor and  $cx$  the total concentration of inhibitor. Then

$$a = cx - n \frac{x - x_0}{m}.$$

If  $k$  is the dissociation constant, the equilibrium will be expressed through the following formula

$$x_0^m a^n = k \frac{x - x_0}{m}. \quad (17 a)$$

If  $c$  is large, i.e. inhibitor is present in excess,  $x_0$  will increase with  $x$  for very small  $x$  values, pass through a maximum, and then decrease. The distribution curve will in fact be beautifully fitted by the figures given by Bawden (7). His table 8 (p. 120) is quoted.

Table XIII.

(From Bawden: Plant Viruses.)

Effect of dilution on the infectivity of mixtures of tobacco mosaic virus and sera.

Dilution	Average number of lesions per leaf		
	Saline control	Normal serum	Tobacco mosaic virus antiserum
1/1	240	24	2
1/5	210	40	4
1/25	52	38	9
1/125	29	18	9
1/625	13	5	6

It is obvious, that a distribution curve of this type is incompatible with the figures in table XII. If, however,  $c$  is approximately 1 it is possible to find a form of function (17 a), which can be fitted to the experimental data. This will be the case if the inactive complex consists of one virus particle and one inhibitor molecule. The following dissociation formula is obtained.

$$x_0 = \frac{k}{2} \left( \sqrt{1 + \frac{4x}{k}} - 1 \right). \quad (18)$$

The expected frequencies of takes can be calculated according to the formula (12) if  $x_0$  is substituted for  $x$ . As  $p$  in that formula is not known, the absolute value of the dissociation constant cannot be calculated from the best fitting function of type (18). We have

$$p x_0 = \frac{p k}{2} \left( \sqrt{1 + \frac{4 p x}{p k}} - 1 \right). \quad (18a)$$

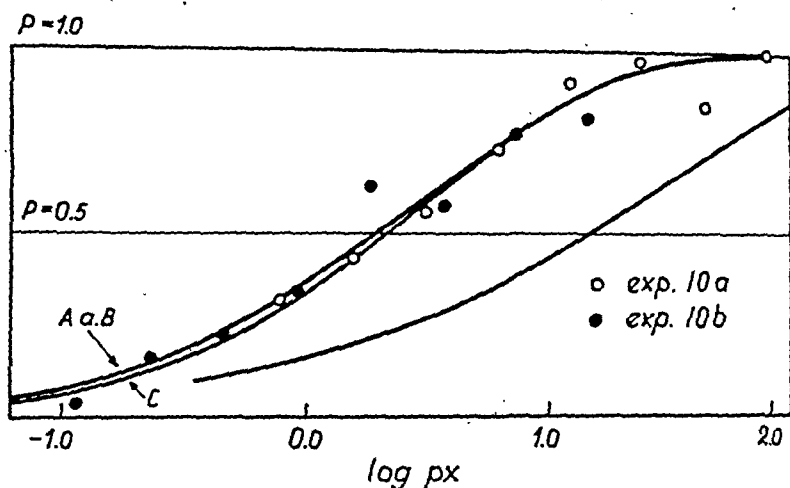


Figure 4. Probability of takes. Curves A—C = distribution according to dissociation formulas A—C. Curve to the right = representation of the function  $P = 1 - e^{-K \cdot \sqrt{x}}$ . Experiment 10.

It is therefore, possible to find only a value of  $pk$  that fits the experiment. This was found to be  $pk = 0.4$ . The corresponding distribution curve is found in fig. 4 (curve A) together with the experimental data. The agreement is good.  $\chi^2$  total was calculated to 15.27, corresponding to a probability of identity of 0.2—0.3. As all active particles in this case must be single molecules the probability of single molecule infections will be  $p x_0 e^{-p x_0}$ . The expected percentage of single molecule infections is recorded in fig. 5 (curve A) together with the data from table XII. The calculated rate of infections of this kind is obviously far too high to fit the experiment.

*Alternative B.* It will be shown later that the molecules of the virus protein are in all probability rodshaped. It is, therefore, likely that

association of molecules, if occurring, would be principally end to end as usually happens with molecules of that shape. If we denote the ends of a molecule  $a$  and  $b$  and suppose that the intermolecular affinities go from  $a$  to  $b$ , we can signify a chain of molecules with the symbol  $ab-ab-ab$ . In this chain all bonds are of the same kind, and in case of a dissociation they will all have the same chance to break, i.e. the dissociation constants of 1, 2, 3, etc. order would be equal. Let  $x_n$  signify the concentration of chains containing  $n$  molecules. If  $k$  be the dissociation constant we have

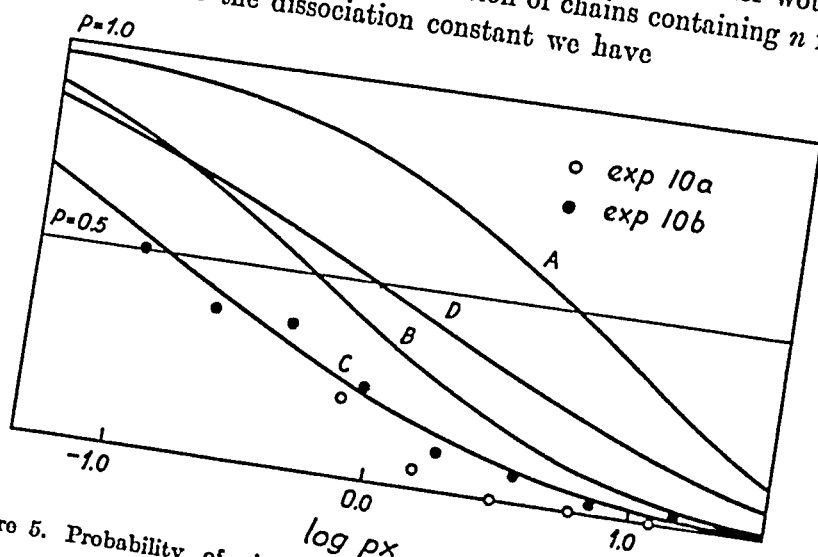


Figure 5. Probability of single molecule infections. Alternatives A—D. Experiment 10.

$$x_2 = x_1/k$$

$$x_3 = x_1 x_2/k = x_1^2/k^2$$

$$\dots \dots \dots$$

$$x_n = x_1^n/k^{(n-1)}.$$

Let  $x$  be the total concentration of virus and  $x_0$  the concentration of aggregates and we have

$$x = x_1 + 2x_1^2/k + 3x_1^3/k^2 + \dots + nx_1^n/k^{(n-1)} + \dots$$

$$x_0 = x_1 + x_1^2/k + x_1^3/k^2 + \dots + x_1^n/k^{(n-1)} + \dots$$

This leads to

$$x = x_1 : (1 - x_1/k)^2 \quad (19 a)$$

$$x_0 = x_1 : (1 - x_1/k). \quad (19 b)$$

And finally

$$x_0 = \frac{k}{2} \left( \sqrt{1 + \frac{4x}{k}} - 1 \right) \quad (19)$$

$$x_1 : x_0 = x_0 : x. \quad (19c)$$

The formula for calculation of the number of active particles will, thus, be exactly the same as the function (18), and the distribution curve consequently identical with curve A fig. 4. The number of single molecule infections will, however, be different. According to function (19c) the ratio of such infections will be  $\frac{x_0}{x} p x_0 e^{-p x_0}$ . The corresponding curve is found in fig. 5 (curve B). Although this alternative gives a lower rate of infections of this kind it is still too high to fit the experiment.

*Alternative C.* If on the other hand the intermolecular bonds go from *a* to *a* and from *b* to *b*, so that a chain of molecules would be signified by the symbol *ab—ba—ab—ba*, there will be two different kinds of bonds with different dissociation constants. Chains of a certain odd number of molecules will all be of the same kind with one free *a* pole and one free *b* pole. Chains of an even number of molecules will belong to either one of two types, with two free *a* or two free *b* poles. Let the concentration of the different kinds of aggregates be  $x_1, (u_2 + v_2), x_3, (u_4 + v_4)$ , etc. Let further the number per unit volume of free poles be  $F_a$  and  $F_b$ , and the number of bonds  $B_a$  and  $B_b$ , and we have

$$F_a = x_1 + 2u_2 + x_3 + 2u_4 \cdots F_b = x_1 + 2v_2 + x_3 + 2v_4 \cdots$$

$$B = v_2 + x_3 + 2v_4 + 2x_5 + \cdots B_b = u_2 + x_3 + 2u_4 + 2x_5 + \cdots$$

The rate of dissociation will be proportionate to  $B_a$  and  $B_b$ . The rate of association will be proportionate to the number of possible combinations of free poles, i.e. to  $F_a^2/2$  and  $F_b^2/2$ .<sup>1</sup> Letting the dissociation constants be  $k_a$  and  $k_b$ , we get the following system of equations,  $x_0$  and  $x$  having the same signification as before:

<sup>1</sup> The number of possible combinations is  $\frac{F(F-1)}{2}$ . The above approximations can be made as  $F_a$  and  $F_b$  have large numerical values.

$$\begin{cases} 2k_a B_a = F_a^2 \\ 2k_b B_b = F_b^2 \\ F_a + F_b = 2x_0 \\ 2B_a + F_a = 2B_b + F_b = x. \end{cases}$$

This leads to

$$x_0 = \frac{k_a}{4} \cdot \left( \sqrt{1 + \frac{4x}{k_a}} - 1 \right) + \frac{k_b}{4} \cdot \left( \sqrt{1 + \frac{4x}{k_b}} - 1 \right). \quad (20)$$

The deduction of the number of single molecule infections can be made in the following way. Using the same symbols and remembering that the same chain with an odd number of molecules is formed regardless whether a single molecule is added to a  $u$ - or a  $v$ -molecule, we have the following series of equilibriums:

$$u_2 = \frac{x_1^2}{2k_a}, \quad v_2 = \frac{x_1^2}{2k_b},$$

$$x_3 = \frac{x_1 v_2}{k_a} + \frac{x_1 u_2}{k_b} = \frac{x_1^3}{k_a k_b},$$

$$u_4 = \frac{x_1 x_3}{2k_a} = \frac{x_1^4}{2k_a^2 k_b}, \quad v_4 = \frac{x_1 x_3}{2k_b} = \frac{x_1^4}{2k_a k_b^2},$$

$$x_5 = \frac{x_1 v_4}{k_a} + \frac{x_1 u_4}{k_b} = \frac{x_1^5}{k_a^2 k_b^2}$$

etc.

We get, therefore:

$$x = x_1 + x_1 \cdot \left( \frac{x_1}{k_a} + \frac{x_1}{k_b} \right) + 3x_1 \cdot \frac{x_1}{k_a} \cdot \frac{x_1}{k_b} + 2x_1 \cdot \frac{x_1}{k_a} \cdot \frac{x_1}{k_b} \cdot \left( \frac{x_1}{k_a} + \frac{x_1}{k_b} \right) + \dots$$

$$\begin{aligned} x_0 = x_1 + 1/2 x_1 \cdot \left( \frac{x_1}{k_a} + \frac{x_1}{k_b} \right) + x_1 \cdot \frac{x_1}{k_a} \cdot \frac{x_1}{k_b} + \\ + 1/2 x_1 \cdot \frac{x_1}{k_a} \cdot \frac{x_1}{k_b} \cdot \left( \frac{x_1}{k_a} + \frac{x_1}{k_b} \right) + \dots \end{aligned}$$

This leads to



$$x = x_1 \cdot \frac{1 + \frac{x_1}{k_a} + \frac{x_1}{k_b} + \frac{x_1^2}{k_a k_b}}{\left(1 + \frac{x_1^2}{k_a k_b}\right)^2} \quad (20 a)$$

$$x_0 = 1/2 x_1 \cdot \frac{2 + \frac{x_1}{k_a} + \frac{x_1}{k_b}}{1 - \frac{x_1^2}{k_a k_b}} \quad (20 b)$$

If  $k_a = k_b$  the functions (20), (20 a), and (20 b) will be identical with the corresponding expressions (19), (19 a), and (19 b), as must be the case.

There is an indefinite number of pairs of  $k_a$  and  $k_b$  that will equally well fit the distribution of frequencies of takes observed. There is, however, only one pair of values that simultaneously gives the best agreement with both the total number of takes and the number of single molecule infections. The curves C in fig. 4 and 5 correspond to  $pk_a = 0.03$  and  $pk_b = 1.5$ . There is a fair agreement with the experimental data.  $\chi^2$  amounts to 17.53 with a probability of identity of 0.1—0.2, thus slightly more than in the cases A and B, but still within the limits of a chance variation. Curve C is the only one to fit acceptably with the number of single molecule infections.

*Alternative D.* There is, however, another line to follow in the search for an explanation of the experimental data. If there is a definite variation in the value of  $p$ , i.e. if the groups of test animals are heterogeneous with regard to resistance, there will be a deviation from the normal distribution of takes. Let  $p_1, p_2 \dots p_n$  denote the different  $p$  values in a group of  $n$  animals. One can easily see that the corresponding probability of takes will be

$$P = 1 - \frac{S(e^{-p_n x})}{n} \quad (21)$$

The corresponding distribution curve will be the average of a series of normal curves, provided that all test groups are similar with regard to the average  $p$  value and the variation around it. It is immediately evident that a curve of that type must have a flatter course than the normal curve. As an example a curve of this kind is shown in fig. 3, (curve D). For the construction of this curve,  $\log p$  was chosen as a

measure of the resistance, and it was further assumed that the frequency distribution was of the normal type, the standard deviation being  $\sigma_{\log p} = 0.6$ . This means that in a group of 48 animals the following classification of resistance could be made:

1)	a group of	3	animals with average	$\log (p_n/p) = -1.2$
2)	»	12	»	» = -0.6
3)	»	18	»	» = $\pm 0$
4)	»	12	»	» = + 0.6
5)	»	3	»	» = + 1.2

The resulting curve fits excellently with the experimental data,  $\chi^2$  being but 13.31 with a probability of identity of 0.3—0.5. The probability of single molecule infections will be

$$P_1 = \frac{S(p_n x \cdot e^{-p_n x})}{n}. \quad (22)$$

The corresponding curve is shown in fig. 5 (curve D). In this respect the fit is not good.

It is impossible to draw any definite conclusions on the basis of the above discussion. In important points the reasoning was based upon the theory of single molecule infections. Although there is much in favour of that assumption, it is nothing but a hypothesis, and until further evidence is collected, proper caution must be exercised. In the following discussion it must be remembered that B and C are the only alternatives to exclude each other. Each one can exist in combination with A, or D, or both. Whichever the combination, the alternative D must be expected to play a part, simply for the reason that absence of individual variation would be contrary to all experience. Further evidence to that end can be obtained from table IX. A distribution curve, based upon one of the functions (18), (19), or (20) has a slope that to a certain extent is dependent upon the value of  $pk$ . As  $k$  must be assumed constant for a given virus and a given solvent, the slope will depend upon  $p$ . With decreasing  $p$  the functions (18), (19), and (20) give distribution curves ever more approaching the limit function (23), where  $K$  has the form  $p\sqrt{k}$ . The slope of the curve

$$P = 1 - e^{-K \cdot \sqrt{x}}. \quad (23)$$

represents, therefore, the lower limit of the corresponding distribution curves. Function (23) is shown in fig. 4. The slope of the approximately straight portion is 0.39 per unit of  $\log px$ . Now we have in exp. 8 table IX, the age group  $a$  with a distribution curve, the slope of which is but 0.125. This type of flat curve cannot be explained on the basis of functions (18), (19) or (20) exclusively. Function (21) on the other hand can assume any degree of slope from 0 to that of the normal distribution curve. As a matter of fact we must expect a considerable heterogeneity in age group  $a$ , as it comprises animals from 54 to 92 days old.

A combination of variation in host resistance and aggregation of virus particles seems to be most fit to explain the results in table XII. It is not even necessary to assume a reversible aggregation. For it must be remembered that the frequency figures in the last column of table XII are at least uncertain, considered the small number of observations. A reduction in the values of curve D, fig. 5, with a constant fraction might therefore very well fit the experiment.

It is hard to say whether the observations by Bald on the tobacco mosaic virus could be interpreted in the same way. In that case one must obviously assume the existence of entry points with different degrees of susceptibility on the surface of one and the same leaf. Although this seems *a priori* not unprobable, to the writer's knowledge there are so far no direct observations on this matter.

Table XII can also serve as basis for a further analysis of the incubation time method. Let  $t_x$  denote the incubation period after infection with  $x$  virus particles, and we can assume the following conversion of the formula (7)

$$\frac{1}{t_x} - \frac{1}{t_1} = b \cdot (\log x - \log 1)$$

$b$  having the same numerical value as in (7), but being positive. From table XII the value of  $\frac{1}{t_1}$  was estimated to  $0.055 \pm 0.004$ . Previously the numerical value of  $b$  was calculated to  $0.0547 \pm 0.0008$ . It seems, therefore, very reasonable to assume  $b = \frac{1}{t_1}$  and simplify the formula to

$$\frac{1}{t_x} = b \cdot (\log x + 1). \quad (24)$$

In a sufficiently large group of animals inoculated with equal amounts of the same virus dilution the fractions infected by 1, 2, 3 etc. virus particles will be

$$P_1 = px \cdot e^{-px}$$

$$P_2 = \frac{(px)^2}{2!} \cdot e^{-px}$$

$$P_n = \frac{(px)^n}{n!} \cdot e^{-px}.$$

If  $\bar{y}$  is the average of  $\frac{1}{t}$  through the whole group of animals we have according to formula (24)

$$\bar{y} = b \cdot [P_1 (\log 1 + 1) + P_2 (\log 2 + 1) + \dots]$$

provided, that  $b$  is constant, i.e. all animals of uniform resistance, and that each particle contains only one virus molecule. But

$$P_1 + P_2 + P_3 + \dots = 1 - e^{-px}.$$

Thus

$$\bar{y} = b \cdot (1 - e^{-px}) + b \cdot S \left[ \frac{(px)^n \cdot \log n}{n!} \cdot e^{-px} \right]. \quad (25)$$

Function (25) has two asymptotes. When  $px$  decreases towards 0 the function will approach «from above» the value  $b \cdot (1 - e^{-px})$ . For large  $px$  the first term will assume the value  $b$  and the second term approach «from below» the value  $b \cdot \log px$ . From values of  $px$  of 10 and upwards function (25) will, therefore, be practically identical with function (24). In fig. 6 the theoretical curve is compared with the experimental data from table XII. The agreement is satisfactory.

If the particles in the virus suspension do not consist of single virus molecules but are aggregates of a constant number of  $c$  molecules, formula (25) will assume the form

$$\bar{y} = b \cdot (1 + \log c) \cdot (1 - e^{-px/c}) + b \cdot S \left[ \frac{(px)^n \cdot \log n}{n!} \cdot e^{-px/c} \right].$$

For large  $px$  values this function will have the same value as (25). For small  $px$  on the other hand  $\bar{y}$  will be significantly lower than in case (25). In case of a reversible dissociation of the aggregates with dilution, therefore, no appreciable deviation from the formula (25) can be expected.

In order to compute the influence of variation of resistance upon the incubation time curve one has to consider that  $b$  certainly varies with  $p$ . As long as the type of this covariation is not known, it is

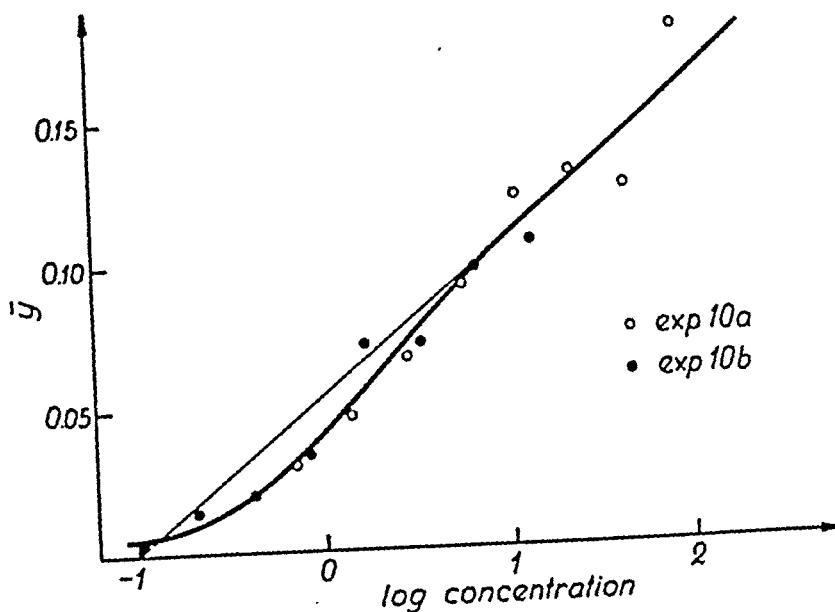


Figure 6. Experimental data fitted to the theoretical incubation time curve. Experiment 10.

futile to speculate over the details of the curve to be expected as the result of such a variation. On the whole, however, it can be constructed like the function (21) as an average of a series of essentially similar curves with different  $b$  and  $p$ . It will, therefore, largely be of the same type although the way in which it approaches the asymptotes and the slope of the rectilinear part will depend upon the covariation of  $b$  and  $p$ .

In the above discussion it was tacitly assumed that more than one virus molecule can enter the same cell, and incite a process of the same course as after as many single hits in different cells. This assumption is by no means self-evident. The phenomenon of interference between different strains of the same virus as exhibited

for instance in yellow fever, must be interpreted as the result of a competition about the susceptible cells. Once a virus particle has entered the cell the affinities are saturated and a superinfection by further virus particles is impossible. As will be shown later there seems to exist no interference in mouse poliomyelitis, indicating that superinfection is possible. From this point of view the reasoning should, therefore, be correct. On certain theoretical grounds to be discussed later one might, however, expect that the course of the infection after a certain number of hits will be different, depending upon the number of cells concerned. At present the experimental evidence towards that end is insufficient, and the analysis cannot be extended further.

The agreement between found and expected as illustrated in fig. 6 does not, of course, prove the validity of the formula (24), but shows that if this function is valid, it can be applied to the average from a group of animals as well. A comparison between fig. 6 and the different types of curves previously discussed reveals the following facts. The point  $px = 1$  corresponds to log dilution 6.96 in the incubation time curve and 6.98 in the distribution curves A, B, and C fig. 4. As these two estimates of the virus concentration are largely independent of each other, the close agreement must be regarded as suggestive evidence of the validity of the formula (24). In the distribution curve D,  $px = 1$  corresponds to log dilution 6.56. This figure is, however, not compatible with the incubation time titer as being based upon different assumptions, and the numerical values now mentioned cannot serve as an evidence in favour of one of the alternatives A—C.

The 50 p. c. point corresponds to log  $px = + 0.24$  in curves A and B and to log  $px = + 0.29$  in curve C. In conformity with these figures the average from a number of titrations listed in table XI was calculated to  $+ 0.277$ .

According to the above discussion the 50 p. c. endpoint titer as a measure of the activity is decidedly unsatisfactory. Its relationship to the actual virus concentration is dependent not only upon the resistance of the test animals but also upon the physical state of the virus particles. The incubation time method on the other hand seems to be largely independent of the latter condition and, therefore, to give a more reliable information about the virus content. In the

present case, therefore, it seems to be more logical to connect the definition of an infective unit with the latter method. Consequently the following definition is formulated.

**DEFINITION.** *One MID of the encephalitogenic strains of mouse poliomyelitis virus contains a number of  $\frac{1}{p}$  virus molecules,  $p$  being the resistance factor of the probability function. The activity of a given virus solution is the number of MID per ml.*

For calculation of the activity one has to use the formula (24). Let  $A$  be the activity. The standard volume of the inoculum is 0.02 ml. Consequently we have

$$\bar{y}_0 = b \cdot (\log 0.02 A + 1)$$

$$\log A = \frac{\bar{y}_0}{b} + 0.7 \quad (26)$$

$\bar{y}_0$  denoting the reciprocal of the harmonic mean of the incubation periods obtained after inoculation of the undiluted material and  $b$  having the value 0.0547. All titer values recorded in the following were calculated according to this formula.

\* \* \*

It was previously pointed out that the correlation between the amount of virus inoculated and the length of the incubation period led to the assumption that the rate of multiplication of virus was "overlogarithmic". An attempt to visualize the mechanism of this process was made on the basis of the following reasoning.

A logarithmic rate of multiplication will be the result if each virus particle through branching or otherwise gives rise to a constant number of daughter particles and the generation period is constant throughout the growth phase. Let the number of daughter particles be  $m$ , the generation period  $t$ ,  $y$  the number of descendants of one single virus particle at the time  $x$ , and we have

$$y = m^{x/t}$$

or

$$\log y = \frac{\log m}{t} \cdot x.$$

*Significance of Persisting Positive Thymol Reaction after Apparent Clinical Restitution.*

Kunkel & Hoagland (13) applied the thymol test in convalescence after acute hepatitis and found it to be positive in 67 % of 27 cases with persisting symptoms (the nature of which is not stated). Of 30 without symptoms only 7 % had a high thymol value.

About 18 % of the cases of acute hepatitis examined at the Hospital of the Rockefeller Institute, N. Y. had a clinical relapse during convalescence. In all these cases there was an increase in the thymol value one to three weeks after the relapse set in. In some instances the thymol value was higher than at the primary attack, and in some the increased values persisted up to six months after other objective signs of the relapse had subsided.

For the purpose of examining the significance of a persistently high thymol value, 12 of the patients discharged as apparently recovered, while still having a high thymol value, were examined subsequently (fig. 4).

Special interest attaches to the curves of five of the patients because, in contrast to the others who were after-examined, they still presented both subjective (lassitude, nausea, vomiting) and objective symptoms (jaundice [sub-jaundice]) of defective restitution.

As these cases also seem to be of particular interest in the question of the significance of a persistently positive thymol reaction, their data are given below in brief outline (Th. = thymol value, TA. = Takata Ara, II = icterus index).

No. 1 (♀ b. 14/12-20) (Curve 1) on discharge had Th. = 0.27, TA. negative, II. 8. The after-examination shows considerable lassitude, occasional epigastric oppression and nausea, but no jaundice. Four weeks after discharge, Th. was 0.67, TA. positive.

No. 2 (♀ b. 1/5-13) on discharge had Th. 0.42, TA. weakly positive, II. 7. Constant lassitude since discharge. In the period three to five weeks after discharge had a temperature, about 38°, sometimes nausea and epigastric oppression. At this juncture there is marked fluctuation in Th. to 0.75, TA. as on discharge, weakly positive, II. 12. Fourteen days later a subjective improvement, temperature normal, and a considerable fall in Th. to 0.35, TA. still weakly positive, II. only 6.

No. 3 (♀ b. 24/3-24) on discharge had Th. 0.17, TA. negative, II. 5, but in the ten weeks prior to the after-examination had two relapses with the symptoms lassitude, nausea, vomiting, pale faeces, dark urine. At the after-examination the only complaint is tiredness. Th.



cells, however, would be larger in the latter case, i.e. the inhibiting influence less pronounced, and one would accordingly expect a more rapid multiplication. This is in full agreement with the results in table V, illustrated in fig. 1, these experiments showing that application of large inocula gave disproportionately short incubation periods. — If on the other hand the ratio susceptible cells / resistant cells decreases, as must be the case in adult mice, the rate of multiplication of virus would decrease, in accordance with the experimental data (tables VIII and IX).

In the formula (27)  $m$  can be said to represent the type of biological or chemical process, significant for the propagation of the virus in question, while  $t$  is a measure of the rate of multiplication, i.e.  $m$  is a characteristic of the virus,  $t$  one of the host. An inhibitory effect from the side of the host would be most likely to cause a change of  $t$ , while  $m$  would be unaffected.

Suppose that the susceptible cells are evenly distributed over a sphere, the radius of which is unit. In the centre the inoculum is deposited, spreading over a concentric sphere with the radius  $r$ . From the site of inoculation the virus migrates evenly in all directions, the rate of migration being  $v$ . The number of intact cells at the time  $x$  after inoculation will then be proportionate to  $1 - (r + vx)^3$ , and the rate of inhibition will be a function of this expression.  $r$  is an arbitrary constant, dependent upon the conditions of the experiment and  $v$  might be a constant or be a function of  $r$  and  $x$ . Let  $t$  be the generation time in the case, that all susceptible cells are invaded, and the rate of multiplication can be expressed as follows.

$$\frac{dy}{dx} = \frac{\log m}{t + f[1 - (r + vx)^3]} \cdot y. \quad (28)$$

At present it is impossible to give any hints as to the actual form of this function. If, however, the rate of inhibition is a linear function of the number of intact cells and the rate of migration of the virus is constant, the formula could be given the following form

$$d(\log y) = (C_0 + C_1 x + C_2 x^2 + C_3 x^3 + \dots) \cdot dx \quad (28a)$$

$C_0, C_1, C_2$ , etc. being constants.

According to the reasoning on page 36 the formula (24) leads to the following growth equation

$$\log y = \frac{bx}{1-bx} \quad (29)$$

By differentiating we get

$$d(\log y) = \frac{b}{(1-bx)^2} \cdot dx = (b + 2b^2x + 3b^3x^2 + \dots) \cdot dx \quad (29a)$$

In the formulas (28a) and (29a) the series of coefficients  $C_0, C_1, C_2, \dots$  and  $b, 2b^2, 3b^3, \dots$  are rapidly converging towards 0. For small values of  $x$ , therefore, all terms from a certain  $C_n x^n$  are in-

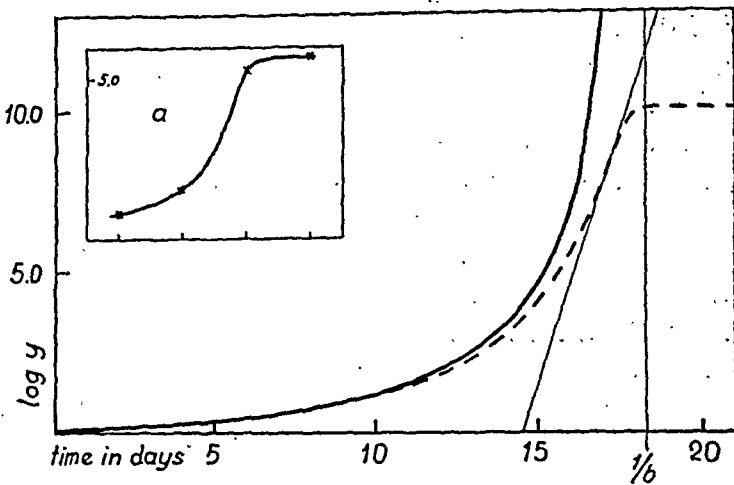


Figure 7. Multiplication of virus, theoretical curves. Inserted (a) experimental data from Theiler and Gard.

significant. It is, therefore, possible to identify (28a) with (29a) for small values of  $x$  by arbitrarily making a number of the coefficients  $C_n$  identical with the corresponding ones in the formula (29a). The coefficients  $C_n$ , however, are functions of the five unknown quantities in the formula (28), and therefore not more than five of them could be arbitrarily chosen. Thus it is possible to find values of the constants such as to give  $C_0 = b$ ,  $C_1 = 2b^2$ ,  $C_2 = 3b^3$ ,  $C_3 = 4b^4$ , and  $C_4 = 5b^5$ , while the rest of the coefficients  $C_n$  might differ from the corresponding  $nb^n$ . Formula (28a) will then be identical with (29a) at least up to the point, where the term  $6b^6x^5$  begins to be significant.

The growth curve corresponding to the formula (29) is shown in fig. 7 (the heavy line). For  $x = \frac{1}{b} \log y$  as well as  $\frac{d(\log y)}{dx}$  are in-

finite. This can hardly agree with the experimental data. On the contrary it was previously pointed out that the concentration of virus never exceeds a certain limit. For  $x = \frac{1}{\bar{y}}$ , therefore,  $\log \bar{y}$  should assume a certain well defined finite value. It is also obvious that the rate of multiplication of virus must have a finite value. On page 54 it was pointed out that the method used for calculation of the incubation period gives but approximately correct figures. From this fact it is evident that the formula (29) gives only an approximation of the real growth function. The corrections to be instituted on behalf of the approximated calculation of the incubation period lead to a displacement of the curve towards larger  $x$  values. The dotted line in fig. 7 is an attempt to illustrate the probable course of function (28). The flex tangent (fine line) denotes the maximum rate of multiplication. When this point is reached all susceptible cells are invaded and multiplication can proceed only till the virus concentration of each cell has reached the limit. In fig. 7 a further diagram is inserted, based upon an experiment by Theiler and Gard (*Jour. Exp. Med.* 72, p. 60, table V), in which the virus content of the brain was determined at intervals after inoculation. Although no direct comparison as to numerical values is possible, it is obvious that the multiplication of virus really follows a curve of the general type now described.

This discussion was not aimed at solving the problem. The writer's intention has been to present a general line of thought along which the solution might possibly be found. As to the nature of the inhibitory factor and the transmission of the effect from one cell to another nothing but a guess can be advanced. It might be a substance produced in the cells and excreted, or it might be conceived as a neurally transmitted mutual control of the metabolism, a sort of trophic tonus.

## CHAPTER V.

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### Purification of neurovirus.

When Stanley (142) in the year 1935 obtained a crystallizing protein, showing all the characteristics of tobacco mosaic virus, a new epoch in the history of virology had started. Not that the purification of viruses with the aid of chemical methods was a new idea. Attempts to that effect had been made for about a decade. Neither was Stanley's protein the first pure virus preparation, elementary bodies of vaccinia being prepared in more or less pure state by MacCallum and Oppenheimer in 1922 (101). Stanley, however, purified a virus with elementary particles of such a small size as to be classified as molecules. His investigations proved that this virus in pure state acted as a molecular chemical compound, as a protein. Viruses were regarded as minute microorganisms and as such in some mysterious way standing above the laws of chemical substances. On that account Stanley's result was felt like a shock. Later, however, it was realized that it was not conclusive evidence against the vitalistic theory.

Stanley's work incited numerous attempts to purify viruses of different kinds, principally plant viruses. His results were confirmed, and using the same method of purification — repeated differential precipitation with ammonium sulphate at controlled pH — Bawden and Pirie (8, 9, 10) were able to obtain in pure state the cucumber viruses 3 and 4 and the viruses of latent mosaic and bushy stunt. In the case of less stable viruses, however, this procedure was not satisfactory, as the material was largely inactivated during the purification process. Attempts were made, therefore, to find less drastic methods. MacCallum and Oppenheimer had found that the virus of vaccinia could be purified by means of differential centrifugation. This method was later successfully used by Ledingham (97) and further elaborated by Parker and Rivers (114). At the same time the

quantity high speed centrifuge had been largely improved by Gratia (55) and especially by Beams and Pickels (11). It was now possible to subject comparatively large quantities of material — up to 100 ml — to centrifugal fields of more than 50 000 times gravity at a low temperature, the latter not the least important when treating unstable organic material. The new equipment was tested in purification experiments and proved to yield excellent results. Thus it was possible to obtain in pure state the viruses of tobacco ring spot and severe etch and the cucumber virus 1. (144).

Animal viruses, usually being less stable than plant viruses, have proved more difficult to purify. The chemical methods have generally not yielded satisfactory results. By means of differential centrifugation, however, supposedly pure preparations of the viruses of vaccinia, Rous' sarcoma (25), Shope's rabbit papilloma (12), and equine encephalitis (162) have been obtained.

Janssen (74) elaborated for the virus of foot and mouth disease a purification procedure that differs principally from the usual methods. Adsorption and elution as used in the enzyme chemistry had frequently been tested in experiments on viruses. It was, however, found a matter of difficulty to elute the virus without destruction of the activity. Janssen, therefore, aimed at a method by which the adsorbent could be manufactured in the reaction mixture, centrifuged off and redissolved, thereby making the virus free. He found that a virus containing fluid, saturated at room temperature with calcium sulphate, on addition of 20 p. c. alcohol or a mixture of alcohol and ether, formed a precipitate containing all the activity and but little extraneous material. This precipitate could be redissolved in water and the process repeated deliberately. The final preparation consisted chemically of a nucleoprotein and was considered by Janssen to be pure virus. He also attempted later to purify the virus by means of differential centrifugation (75).

Attempts at purification of the virus of poliomyelitis have been made now and again. Howitt (72) found that infectious spinal cord could be defatted with ether and that a considerable amount of extraneous proteins could be removed from the remainder by precipitation with basic lead acetate, without much loss of activity. Clark (23, 24) and his collaborators concentrated virus containing extracts by means of vacuum distillation, removed the excess of salts in dialysis and precipitated the virus with ammonium sulphate.

They found, that the virus was completely precipitated in 50 p. c. saturated solution but remained largely in the supernatant at 30 p. c. saturation. If the material had been extracted with ether before the precipitation, the virus was practically insoluble in distilled water. They found finally, that the virus could be concentrated by means of centrifugation at high speed.

Rhoads (126) had shown that the virus could be adsorbed on aluminum hydroxide. Sabin (128) found that the activity could be eluted in quantity from the precipitate with dilute disodium phosphate solution. After subsequent dialysis and vacuum distillation he obtained a preparation giving negative tests for protein and ten times as active as the starting material. Behrens and Barker (13) accomplished a partial purification by means of repeated isoelectric precipitation at pH 4.8—5.0 with 0.01 m citric acid.

Brown and Kolmer (16) used differential precipitation with ammonium sulphate according to Stanley and obtained a solution practically retaining the original activity. It flocculated sharply at pH 4.0. The precipitate could be dissolved in water and reprecipitated at the same pH. The final solution contained but 0.01 p. c. protein.

## I. Neurovirus from mice.

The principal difficulty involved in the purification of brain and cord suspensions, is the presence of large amounts of lipid matter. These substances are easily emulsified even in dilute protein solutions. On account of blocking they make filtration of the extracts practically impossible. They have a low specific gravity and cannot, therefore, be removed by centrifugation. This difficulty was encountered by Beard and his collaborators (37) purifying the virus of equine encephalitis from chick embryos. They observed serious disturbances of the purification process unless the brain and cord were removed from the embryos before preparation of the extracts.

If a brain suspension is shaken with half the volume of ether, the mixture forms a homogeneous emulsion. On standing, however, it soon separates into two or three distinct layers: a top layer, consisting of ether and ether soluble substances, a middle layer containing lipoids and lipoproteins, insoluble in ether and water, and an aqueous bottom layer with the water soluble substances. This aqueous solution, however, still contains large amounts of particulate matter of low

specific gravity, making further purification by means of differential centrifugation impossible.

In an attempt at concentration and partial purification of poliomyelitis virus from sewage and stools the present writer employed precipitation with ammonium sulphate (46). With the aim to obtain the virus fraction in an easily separable form the precipitation was made in the presence of ether. The top layer was found to contain the whole precipitate. When the top disc was dialysed or extracted with water, it was observed that the extract remained water clear as long as a sufficient amount of ether was present but turned opalescent or turbid as soon as the ether was removed.

This observation was used by Gard and Pedersen (50) in purification experiments on mouse poliomyelitis virus from infected mouse brain. A brief account of these experiments has previously been published. In the following they will be described in detail together with the subsequent work.

### Preparation of extracts.

First it was attempted to determine the most favourable conditions for extraction of the tissue.

*Experiment II.* 18 brains were ground with sterile sand and 15 ml of distilled water. 2 ml portions of the heavy suspension were thoroughly mixed with 8 ml of buffer solution (ionic strength  $\approx 0.1$ ). After 2 hours at  $+4^{\circ}\text{C}$  the mixtures were centrifuged at low speed, the supernatants removed and diluted 1:100 and 1:10 000. Of each dilution 0.02 ml amounts were inoculated intracerebrally to groups of 5 mice. The results are summarized in table XIII.

Table XIII.

Influence of pH at the extraction of virus containing nerve tissue.

Buffer	pH of extract	titer of extract = log A
Acetate .....	5.1	7.8
Phosphate .....	6.1	7.4
" .....	7.1	7.7
Borate .....	7.8	7.9
" .....	9.0	8.3
" .....	9.7	8.1
Dist. water .....	7.3	8.1

The differences observed are not statistically significant. There is, however, a certain trend towards an optimal pH at about 9. This coincides fairly with the range of maximum stability of the virus. The differences might, therefore, well be regarded as a pH effect on the stability rather than the solubility of the virus.

In similar experiments the effect of different salt concentration of the diluent — from 0 to 2 m sodium chloride solution — was studied. No significant differences were observed. It was found, however, that the coarse material of the suspensions showed a maximum sedimentation velocity in the range between 0.025 and 0.075 m NaCl.

The quality of the extracts was to a certain degree dependent upon the quantity of diluent used. If 10 p. c. suspensions were left to settle over night in the cold room, the bottom layer of coarse material usually amounted to more than 50 p. c. of the total volume, and the supernatant was very turbid. In 5 p. c. suspensions the bottom layer was less than 20 p. c. and the supernatant much clearer. Further dilution had no significant effect.

*Experiment 12.* The completeness of the extraction was studied in the following experiment. 500 brains were ground with sand and made up with diluent to a total volume of 3 500 ml. After settling over night in the cold, 2 750 ml of supernatant was siphoned off, and further diluent added to the remainder up to the original volume of 3 500 ml. A second extract of 3 000 ml was collected. The activity of the extracts was tested and found to be  $\log A = 6.35$  and 5.36 respectively. Thus, the first extract contained 90 p. c. of the total activity.

In accordance with the above experiments the crude extracts were prepared as follows. The brains were thoroughly ground with sand and mixed with a diluent consisting of 0.05 m sodium chloride solution to which was added phosphate buffer of pH 8.0 to an ionic strength of  $\mu = 0.001$ . The total volume was adjusted to give a 5 p. c. suspension, calculated on brain wet weight. The mixture was allowed to settle in high glass cylinders over night in the cold room. The supernatant extract was then siphoned off and the remainder discarded.

### Removal of the lipoidal constituents.

In the crude extracts rather large quantities of material were sedimentable at high speed centrifugation. Part of this sedimented also at low speed, and during repeated fractionation in the centrifuge



an increasing amount of coarse material could be removed. When analysed in the ultracentrifuge the purified remainder, however, proved to be inhomogeneous, without any distinct sedimentation boundaries, regardless of the number of fractionations. There seemed to be no end to this purification procedure. If differential centrifugation was to be employed, which from several points of view was favourable, the crude extracts must obviously undergo some initial treatment in order to remove the mass of lipoidal matter.

Since desiccation in vacuo destroys the activity, extraction of the dried material with benzene and other lipoid solvents was out of the question. As already mentioned separation with ether according to the three-layer-method of the aqueous extracts led to a considerable purification, although enough lipoids were left in solution to make further purification by means of differential centrifugation a tiresome business. It was, therefore, attempted to get more suitable extracts by means of enzymatic digestion of the macerated brains.

*Experiment 13.* In four experiments infected brains were ground with sand and small amounts of water. Parts of the heavy suspensions were mixed with 3 or 4 volumes of equal parts of pancreatic juice and glycerin. A set of controls were mixed with 50 p. c. glycerin and their pH adjusted to that of the reaction mixtures. The samples were kept at 37° C for 16—36 hours. They were then centrifuged at low speed and the supernatants titrated in mice. The results of these tests are summarized in table XIV.

Table XIV.

Influence of digestion with pancreatic juice on the activity of infected mouse brains.

Time		Titer = log A	Difference
36 hrs. ....	{ Digest ....	8.09	+ 0.79
	{ Control ...	7.30	
16 hrs. ....	{ Digest ....	7.49	+ 0.65
	{ Control ...	6.84	
24 hrs. ....	{ Digest ....	7.15	+ 0.39
	{ Control ...	6.76	
24 hrs. ....	{ Digest ....	8.27	+ 0.62
	{ Control ...	7.65	

In all cases the digest had a higher titer than the corresponding control. The mean difference  $0.61 \pm 0.146$  is statistically significant.

The digestion obviously did not inactivate the virus. Whether on the other hand the apparent activation should be accounted for by a preservative effect, an actual activator in the pancreatic juice, or the destruction of an inhibitor in the brain tissue remained unsettled. There are, however, facts in favour of the two latter alternatives. The digested material was subjected to differential centrifugation but proved to be even less suitable than untreated extracts.

*Experiment 14.* Several other procedures were tested. In preliminary experiments it was found, that a maximum of flocculation occurred at about pH 4.5. The following experiment was set up. 9 brains were ground with sand and 15 ml of distilled water. The suspension was centrifuged to remove sand and coarse particles. The supernatant was divided into 4 portions, to each of which was added 1 volume of buffer solution of ionic strength  $\mu = 0.1$ . The mixtures were centrifuged at low speed, the supernatants poured off and sampled for pH and nitrogen determinations. 1 ml of each supernatant was neutralized against phenol red, made up to 10 ml, and titrated in mice. The results are summarized in table XV.

Table XV.  
Isoelectric precipitation.

Buffer	Supernatant	pH	mg N per ml	titer
Acetate .....	almost clear	4.07	0.422	3.41
" .....	clear	4.39	0.417	2.88
" .....	"	4.57	0.407	2.83
Phosphate .....	turbid	7.19	0.789	6.61

From this it is evident, that the total activity is precipitated together with approximately 50 p. c. of the nitrogen. Clarification by this means was therefore not practicable. — In a second experiment the properties of the precipitate were studied.

*Experiment 15.* To a measured amount of extract acetate buffer was added to give a final pH of 4.5. The mixture was centrifuged at low speed, the supernatant poured off and neutralized. The precipitate was suspended in phosphate buffer of pH 8.0, centrifuged and made up to the original volume. Both fractions were titrated in mice together with an untreated control. The titers were: precipitate 8.00, supernatant 4.34, and control 8.00. Thus the activity was completely recovered from the precipitate. The suspension was milky and presented the same difficulties at centrifugation as did the original extract.

Janssen's purification method was tested. The virus was, however, rapidly inactivated in the concentrations of alcohol used. At a concentration of 5 p. c. alcohol the loss of activity amounted to only 10 p. c. but the precipitate formed contained less than 1 p. c. of the total activity. Other adsorbents were tested as well. Floridin was selected on that account, that it can be easily removed by sedimentation without centrifugation. None of the grades tested, however, adsorbed significant quantities of either virus or impurities.

Finally precipitation with ammonium sulphate was studied.

*Experiment 16.* To portions of 10 p. c. brain suspensions saturated ammonium sulphate solution was added to give final concentrations of 40, 30, and 20 p. c. saturation. 1/10 by volume of ether was added and the mixtures thoroughly shaken. They were then centrifuged at 3 000 r. p. m. for 15 minutes. In the two higher concentrations a top disc had formed and the mother liquor was clear, respectively almost clear. In 20 p. c. saturation there was no top layer, a precipitate had settled, and the supernatant was turbid. The mother liquors as well as the two top discs were made up to 100 times the original volume of extract with saline solution. The precipitate of the 20 p. c. solution was discarded. The diluted samples were titrated in mice with the results tabulated in table XVI.

Table XVI.

Precipitation with ammonium sulphate.

Salt concentration	Titer of	
	mother liquor	precipitate
40 p. c. saturation .....	5.08	8.39
30 p. c.       " .....	7.85	7.97
20 p. c.       " .....	8.65	—

At 40 p. c. saturation 99.95 p. c. of the virus was precipitated, at 30 p. c. saturation about 4/7, and at 20 p. c. only an insignificant amount.

The method for initial purification of the crude brain extracts finally adopted was as follows. The suspension was mixed with 1/2—2/3 by volume of ether and thoroughly shaken. The mixture was kept in a separatory flask in the cold room for 4—5 hours. The bottom layer was collected, 2/3 by volume of saturated ammonium sulphate solution was added, and the mixture left to settle in the cold during 4—5 hours. Then the water clear bottom layer was poured

off and discarded. The top disc, light greyish red in colour, was suspended in 3 volumes of distilled water, saturated with ether, and left in the cold room over night. The bottom layer, faintly yellow and opalescent, was collected, and the top layer resuspended in ether containing distilled water. The second extract was colourless and clear. The extraction was repeated twice more. The pooled extracts were run twice through a De Laval industrial separator used as clarificator with a centrifugal field of about 5000 times gravity. The solution was now free of ether and practically water clear. It was then concentrated by means of ultrafiltration as described by Seibert (137). In repeated tests it had been controlled, that no loss of activity occurred during filtration.

Usually lots of 500 mouse brains were prepared, yielding about 3000 ml of crude extract. The extract of the ammonium sulphate precipitate as a rule amounted to about 2000 ml, and was concentrated to 100 ml by ultrafiltration. The final product could easily be subjected to further purification by means of differential centrifugation without the disturbances previously mentioned.

### Differential centrifugation.

In these experiments a Beam's centrifuge was used. The angle head could hold 16 tubes of 6.5 ml effective volume each. The centrifugal field was about 40 000 times gravity at a speed of 22 000 r. p. m. Before each run the rotor was cooled to  $+4^{\circ}\text{C}$ . At the end of a run of three hours the temperature would usually not exceed  $+6^{\circ}\text{C}$ . During the summer, when the moisture content of the air was high, large quantities of water condensed on the rotor during the transport from the cold room to the centrifuge, which hampered seriously the evacuation of the vacuum chamber. To avoid this the rotor was enveloped in a slip-cover of watertight material with a zipper, that could rapidly be removed immediately before the vacuum chamber was closed. This simple device served the purpose satisfactorily.

#### *Experiment 17.*

a) A 10 p. c. crude suspension of infected brains was spun in two experiments for 1 and 2 hours at 22 500 r. p. m. In both cases a yellowish brown, almost clear pellet had settled. The supernatants were pipetted off and the pellets resuspended in the original amount of diluent. Both fractions, supernatants and precipitates, were etherized, stored in the icebox till the following day and titrated in mice. The titers were:

1 hour supernatant	4.37	precipitate	5.97
2 hours	4.39		6.08

Thus in both cases about 98 p. c. of the activity was found in the precipitate.

b) In another experiment a crude extract was spun 1 hour at 22 500 r. p. m. The supernatant was poured off and the precipitate resuspended in 1/10 of the original volume. After etherization the concentrate was titrated together with an untreated control. Titer of concentrate: 7.96, control: 6.80. Thus an apparent gain of activity of about 45 p. c. had occurred. The difference was, however, not statistically significant.

c) A 10 p. c. suspension was spun at 11 000 r. p. m. for 1/2 hour. The precipitated material forming a yellowish brown pellet was discarded, the supernatant after etherization titrated together with an untreated control. Titters: supernatant 7.73, control 7.50. In this case also an apparent gain of activity, about 70 p. c., had occurred.

These preliminary experiments showed that the virus under the prevailing conditions sedimented completely in 1 hour in a field of 40 000 times gravity, whereas only insignificant quantities settled during 1/2 hour in a field of 10 000 g. Accordingly the following purification procedure was tested.

*Experiment 18.* A 10 p. c. suspension was spun in an angle centrifuge at 3000 r. p. m. for 1/2 hour. The turbid supernatant was centrifuged at 22 500 r. p. m. for 2 hours. The supernatant was poured off = S1. The pellets were resuspended in diluent and spun at 11 000 r. p. m. for 1/2 hour. The sediment was taken up into diluent to the original volume = B2. The supernatant = S2, after sampling for activity test, was spun once more at 22 500 r. p. m. for 2 hours. The supernatant from this run = S3. The sediment was resuspended and spun at 11 000 r. p. m. for 1/2 hour. The resulting fractions = S4 and B4. All preparations were yellowish in colour and somewhat opalescent. They were titrated in mice together with an untreated control. The result is summarized in table XVII.

From this it is evident, that a great deal of the activity is lost with the fractions B2 and B4, and possibly in increasing amounts as the procedure is repeated. Another matter of interest is the fact that the total of the fractions S1, S2, and B2 shows a gain in activity of about 40 p. c. as compared with the control, whereas S3, S4, and B4 together yield the same amount of virus as did S2, from which they were derived.

The same observation was made in two of the preceding experiments, and seems to be a constant feature of the initial steps of purification. The reason is probably the presence in the crude extracts

Table XVII.

Differential centrifugation of crude brain suspension.

Fraction	Titer	Difference	Rel. conc.	Total
Control .....	7.34		100	100
S1 .....	6.25	- 1.09	8	141
S2 .....	7.38	+ 0.04	110	
B2 .....	6.70	- 0.64	23	
S3 .....	6.03	- 1.31	5	100
S4 .....	7.15	- 0.19	65	
B4 .....	6.86	- 0.48	30	

of one or two inhibitors — one that is not sedimentable and therefore separates from the virus by centrifugation at high speed, and another that is connected with the coarse material. Neither seems to combine with the virus *in vitro*. They are destroyed by digestion with pancreatic juice. The first one originates probably from the blood retained in the brain and may be identical with the neutralizing substance, shown by Olitsky to be present in serum of normal mice. The other inhibitor might have some connection with the tissue immunity; at least it is not present in brains of normal mice. The whole question has a bearing upon the problem of interference between different strains of virus as studied by Jungeblut and Sanders (78). Experiments along this line are carried out and will be reported in another connection. On account of the circumstances now mentioned it was considered inadequate to express the yield in terms of the activity of the crude brain suspension. In the following, therefore, all such figures refer to the virus content of extracts that have been subjected to initial purification.

*Experiment 19.* In order to avoid the heavy losses of activity the technique was somewhat modified in the following experiment. 350 brains were extracted as previously described, yielding 960 ml of crude suspension. This was spun at 11 000 r. p. m. for 1/2 hour. The supernatant = S1 was sampled for activity test and centrifuged at 22 500 r. p. m. for 1 hour. The precipitate was taken up into a total of 84 ml of diluent, allowed to swell in the icebox for 2 hours and then run up-and-down to 11 000 r. p. m. A small white, brittle precipitate had settled. The supernatant was fractionated in the same way twice more and successively brought down to a volume of 10 ml. All supernatants were

clear at the top with increasing cloudiness towards the bottom of the tube. All runs at the lower speed gave precipitates of the same general appearance as the first one. The final solution = S7 was titrated together with S1. The titers were  $S1 = 7.56$ ,  $S7 = 9.30$ , meaning an increase in titer of 55 times with a decrease in volume of 82 times. The yield was 67 p. c. The preparation was analyzed in the ultracentrifuge and proved to be largely inhomogeneous (fig. 9).

As purification by means of differential centrifugation alone seemed to be not feasible, ether-treated brain suspension was tested as starting material.

*Experiment 20.* 350 brains were extracted twice. The pooled extracts were treated with ether as previously described. After dialysis against tap water the extract was centrifuged first in an angle centrifuge at 3000 r. p. m. for 15 minutes and subsequently at 11 000 r. p. m. for 1/2 hour. The supernatant = S1 had a total volume of 760 ml. It was concentrated by ultrafiltration to 220 ml. The originally waterclear extract deposited during ultrafiltration a floccular precipitate, blocking the filter. The concentrate was fractionated 5 times in the centrifuge at alternately 22 500 r. p. m. for 1 hour and 11 000 r. p. m. for 1/2 hour. The final solution = S11 had a volume of 6 ml, was slightly opalescent and almost colourless. A precipitate had been deposited in all low speed centrifugations although in decreasing amounts as the purification proceeded. Two of these precipitates (B3—5) were sampled for activity test, and likewise two of the supernatants from high speed runs (S8—10). The titration results are summarized in table XVIII. As demonstrated the losses of activity are definitely lower and the yield consequently higher than in the preceding experiments. Nitrogen determinations were performed on S1 = 0.351 mg per ml, and S11 = 0.069 mg per ml. Thus the activity was  $8.3 \times 10^7$  MID per mg N and  $4.7 \times 10^{10}$  MID per mg N and the purification consequently 560 fold. — In a second experiment the yield of virus was but 25 p. c. The solution had a titer of 8.81 and the nitrogen content was 0.046 mg per ml. The activity was, therefore  $1.4 \times 10^{10}$  MID per mg N.

Table XVIII.  
Differential centrifugation of ether treated extract.

Fraction	Titer	Rel. conc.	Volume	Yield
S1 .....	7.48	1.0	760	
S11 .....	9.51	107	6	85 p. c.
B3—5 .....	8.02	3.5	6	2.8 "
S8—10 .....	7.71	1.7	25	5.6 "

When analysed in the ultracentrifuge these preparations showed a higher degree of homogeneity, and it was actually possible to

distinguish two main components (fig. 10 and 11). The sedimentation diagrams proved, however, the state of purity to be far from satisfactory. The supposed virus component was estimated to between 6 and 16 p. c. of the total contents of solids. As furthermore, judging from the diagrams, a complete purification by means of differential centrifugation was not feasible, it was decided to add ammonium sulphate precipitation to the purification procedure.

*Experiment 21.* 450 brains were extracted, treated with ether, and precipitated with ammonium sulphate as previously described. The extract of the precipitate was concentrated by ultrafiltration and centrifuged at 11 000 r. p. m. for 1/2 hour. The supernatant was centrifuged at 22 500 r. p. m. for 2 hours. The precipitate from this run was resuspended in 25 ml of distilled water and kept in the cold for 12 hours. It was then centrifuged once more at 11 000 r. p. m. for 1/2 hour and at 22 500 r. p. m. for 2 hours. The final sediment, a minute, faintly yellow, clear pellet was resuspended in 1 ml of distilled water and analysed in the ultracentrifuge. Afterwards it was titrated in mice. The titer was 10.34, corresponding to a yield of 34 p. c.

The sedimentation diagram (fig. 12) showed the presence of three distinct components, which could probably be separated by means of centrifugation. As, however, one of these might be a high molecular globulin, an attempt was made to eliminate it by employing a sharpened fractionation in the initial ammonium sulphate precipitation.

*Experiment 22.* To 2500 ml of ether-treated extract ammonium sulphate was added to 1/6 saturation. The mixture was centrifuged at 3000 r. p. m. The top disc was removed = fraction a. The remainder was mixed with further ammonium sulphate solution to make a total concentration of 1/3 saturation and again centrifuged. The second top disc = fraction b, the clear mother liquor = fraction c. a and b were suspended in distilled water and centrifuged at 3000 r. p. m. after removal of the ether. All fractions were titrated in mice. The result is summarized in table XIX.

Table XIX.

Fractionated precipitation with ammonium sulphate.

Fraction	Volume	Titer	Yield
a .....	210	7.90	21.8 p. c.
b .....	275	8.42	77.4 "
c .....	3500	5.36	0.8 "



The experiment showed that 1/3 saturation was sufficient for complete precipitation of the virus. Fractionated precipitation on the other hand was not recommendable on account of the great loss of activity.

*Experiment 23.* In the next experiment 1100 brains were used. The ether-treated extract was precipitated at 1/3 saturation of ammonium sulphate, the precipitate washed twice with 1/3 saturated ammonium sulphate solution and finally extracted as usual. The concentrate was fractionated 4 times in the centrifuge, at 11 000 r. p. m. for 1/2 hour and 22 500 for 1 hour. The final sediment was resuspended in 1 ml of phosphate buffer solution, pH = 8.0 and ionic strength  $\mu = 0.05$ .

When analysed in the ultracentrifuge this preparation showed but one distinct component with no traces of impurities (fig. 13). The sediment obtained at high speed centrifugation was an almost colourless, clear, gelatinous pellet, easily soluble in distilled water and dilute salt solutions. The solution was clear with a blueish Tyndall cone. The whole preparation was used for determination of the diffusion constant, why activity test and nitrogen determination were not carried out.

### Ultracentrifugal analysis.

In the analysis of the different virus preparations a study of their sedimentation in centrifugal fields proved to be most enlightening. In these studies two different methods of recording were employed.

The so called separation cell (153) is divided by a perforated membrane into an outer and an inner compartment. After the outer, bottom compartment has been filled with test solution the membrane is covered with a thin filterpaper and finally the inner compartment filled as well. This simple device prevents convection between the inner and outer compartment when the centrifuge is stopped, and the contents of these can be sampled and titrated separately. By this means it is possible to study the sedimentation of a biologically active substance even if its concentration is far below the limits of direct observation.

For optical registration Lamm's scale method (94) was used, by which the refractive index and consequently the concentration gradients are directly recorded.

in the syndrome and have published series of operated cases in 1937—1939, the operations being performed respectively by Tengvall, Boggild, Lendorf and Kjærgaard. The 9 cases previously published by Warburg are included in the patient material here presented.

In France, pericardiectomy was performed by Hallopeau as early as 1910. After this, however, the operative treatment of the syndrome appears not to have attracted any particular interest until 1939, when the first operated cases with non-fatal outcome were reported by Santy, Bernheim, Piquet & Galy. In the same year, a thesis was published by Piquet on the syndrome, comprising 59 operated cases gathered from the literature.

In the case of England the same holds true as has been said about France: that some very valuable contributions to the clinical aspects of the syndrome were given at an early juncture, whereas the operative treatment appears to have been slow in attracting a similar interest as it met with in Germany, U. S. A. and the Scandinavian countries.

Now the total number of operated cases amounts to several hundreds. In 1936 the total number of operated cases was reckoned to be 110 (an account given by Smith & Liggett in 1928 comprises the total of 107 cases, but undoubtedly many of these cases have not been clear-cut — or several of them may have been diagnosed erroneously — as valvular defects were present in no less than 29 cases). Since then, the number of operated cases has been increasing markedly. In 1944 the two largest series of cases reported from a single clinic had grown to about half a hundred cases, and in addition numerous smaller series and single observations have been published.

In the literature it is generally agreed that patients suffering from chronic constrictive pericarditis should be treated operatively, but such treatment should be postponed as long as the patient shows any evidence of active infection. Still, Burwell & Blalock think that no particular risk is involved in operation for tuberculous constriction in the presence of active proliferative tuberculous pericarditis — a view that is not shared by Churchill. On the other hand, the operation should not be postponed too long, as the operative risk increases with increasing cardiac insufficiency, especially when the process of calcification appears in the pericardium. Calcification is no contraindication for the operation, however, and, among others, Settergren advocates a greater liber-

Also, concerning this point, Westermann, from Schmieden's clinic, is an ardent advocate of early operative treatment.

In the various statistics the operative results are fairly alike, with a case mortality of above 25 % in connection with the operation, or in the first years after, and 50—75 % cases of recovery or considerable improvement. Thus in Schmieden's 54 cases, in 1944 Westermann found: 36.9 % recovered, 28.3 % considerably improved, 15.1 % died during, or shortly after, the operation, 5.7 % died in the period of after-treatment, and 15 % died later after transitory improvement. Of 46 patients operated on by Beck, 67.4 % recovered, 4.3 % improved, 2.2 % died under the operation, 17.4 % died in the postoperative period, and 8.7 % died later on. These statistics are essentially alike, as to a large extent it is a matter of definition who are to be designated as recovered or considerably improved. Recovery is defined as a state of health in which the patient is able without any particular trouble to resume his ordinary occupation.

Concerning the literature on chronic constrictive pericarditis, further mention is to be made only of various views as to the etiology of the lesion. In the course of time these views have been changing a good deal, and the question is not settled yet. At an early juncture the French school took the etiology to be tuberculous. Later on rheumatic infection was taken to be responsible for the constriction of the heart in most of the cases. Thus, in 46 % of their 107 cases gathered from the literature, Smith & Liggett found the history of rheumatic infection. But, as mentioned before, the material collected by these authors includes so many instances of valvular defects that it hardly may be looked upon as clear-cut. Tuberculosis is mentioned as the second most frequent cause (29 %).

The more recent works fail to confirm that rheumatic infection is particularly frequent in constriction of the heart (White, Warburg). The earlier view of rheumatic infection being particularly frequent in this lesion is due to the circumstance that no sufficiently sharp distinction has been made between pericarditis in general (which most often is of rheumatic origin) and the special form of chronic pericarditis that gives the constriction of the heart; to the latter a rheumatic infection is of no etiological significance. King even goes so far as to state that a history of rheumatic infection should make us sceptic about the diagnosis of constriction of the heart.

Burwell & Blalock take tuberculosis to be the most frequent cause of chronic constrictive pericarditis, stating that 16 of their 19 patients were suffering from tuberculous constrictive pericarditis. In most other materials the frequency of tuberculosis is recorded as being much lower — about 15 %. Some cases have been of traumatic origin, and in a few cases the syndrome has developed after coronary occlusion. Generally, however, the view prevails that in most cases the etiology of the lesion is quite obscure.

### Patient Material.

For further elucidation of the syndrome we have considered it worth while to give a survey of the patients treated for this lesion in the Medical Department B of the Rigshospital, Copenhagen, since Warburg was appointed chief of this clinic.

Our present patient material comprises altogether 33 cases. Of this total, however, 8 have been somewhat uncertain or complicated, leaving thus 25 clear-cut cases of this lesion. The accuracy of the diagnosis has been established in 1 case by the highly typical clinical features of the syndrome. In the remaining cases the accuracy of the diagnosis was established not only by the typical clinical symptoms but also by the operative findings (19 cases), the roentgenographic findings (4 cases) and the autopsy findings (1 case).

The distribution of the cases on the various years of the period here concerned is shown in Table 1.

Table 1.

*Distribution of the present Cases on the various Years.*

1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	$\frac{1}{2}$ — $\frac{1}{3}$ 47
2	0	1	2	2	0	0	1	3	1	3	0	2	6	2

From Table 1 it will be noticed that the first 15 cases are distributed fairly equally over the years of 1933—1944, while in the first 2 years after the cessation of the world war no less than 10 patients were admitted.

This material is made up of 18 men and 7 women.

The age distribution of the material is given in Table 2.

Table XXI contains also the total virus content of the cell, the control as unit, calculated on the basis of the titer values. The previously mentioned deficit of activity is evident, increasing with time. This indicates a loss of virus with the ever closer packed sediment at the bottom of the cell, from where it is not easily resuspended, rather than through adsorption in the filter paper. It should be noticed, that the 45 min. value falls out of line in this series as well.

Table XXI.

Sedimentation constants and total virus content of the cell on the basis of titer values from table XX.

Centrifugation time	Sedimentation constant	Total virus content
30 min. ....	$151 \times 10^{-13}$	1.01
45 " ....	$195 \times 10^{-13}$	0.90
60 " ....	$154 \times 10^{-13}$	0.90
90 " ....	$151 \times 10^{-13}$	0.80
weighted mean ....	$152 \times 10^{-13}$	

The weighted mean of all four values is  $152 \times 10^{-13}$ .<sup>1</sup>

### B. Optical recording.

A few of these experiments were carried out in the oil turbine centrifuge at 21 000 r. p. m., but in the majority of cases an electrically driven "equilibrium" centrifuge was used. The top speed was 18 000 r. p. m., corresponding to a field of 18 800 g. All runs were made at 0° C. The data were recorded and the results computed as usual. All figures were corrected to + 20° C.

In a series of preliminary runs crude suspensions of normal as well as infected brains were studied. The material proved to be very inhomogeneous. Sometimes more distinct components were observed, but these observations usually could not be reproduced and confirmed, the reason for which might have been that the system was very sensitive to small alterations of the salt concentration.

<sup>1</sup> In the preliminary communication the constant was calculated to  $160-170 \cdot 10^{-13}$ . This estimation was based on individual values from part of the runs now reported and must be considered less accurate than the figure given above.

The first more definite result was obtained with preparation II (experiment 19). In repeated experiments reproducible sedimentation diagrams were recorded. Fig. 9 shows one of these. The heavy line represents the concentration gradients actually recorded. The fine dotted line is an attempt to resolve the diagram into its constitutional elements. The material thus seemed to contain four components, three of which were homogeneous, the fourth and major component, however, being largely inhomogeneous. The sedimentation constants

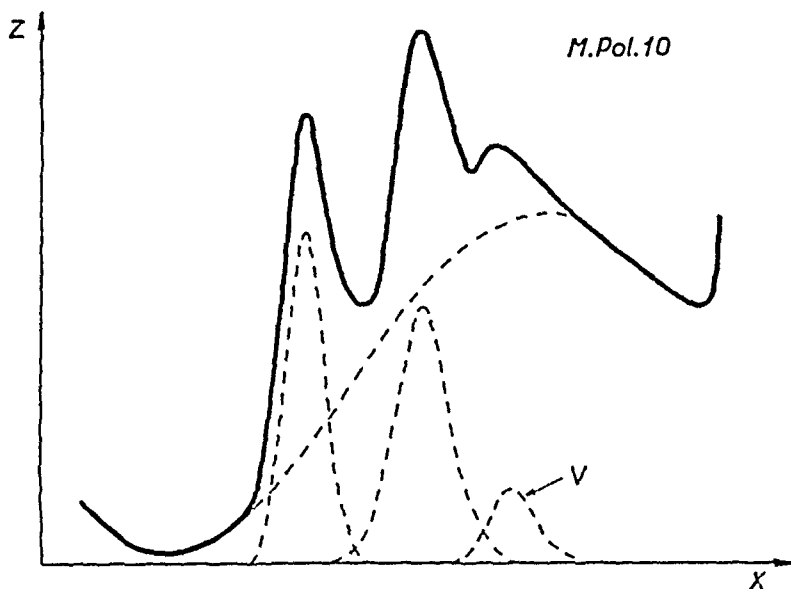


Figure 9. Sedimentation diagram of preparation II.  $V$  = virus component. Centrifugal field 32 000 g. Centrifugation time 40 minutes.

of the distinct components were  $s_{20} = 81, 118, \text{ and } 144 \times 10^{-13}$ . Of these only the latter is in the range where the virus would be expected. In four experiments the values 144, 151, 147, and 157, averaging  $150 \times 10^{-13}$  were found. On account of the uncertainty in interpretation of the diagram no calculations of the concentrations were attempted.

Preparation III (experiment 20) showed when analysed the presence of two main components. The concentration was, however, too low for accurate observations. Through high speed centrifugation and resuspension the material was concentrated 5 times, after which the diagram in fig. 10 was obtained. Of the three main components one is homogeneous with a sedimentation constant  $s_{20} = 151 \times 10^{-13}$ ,

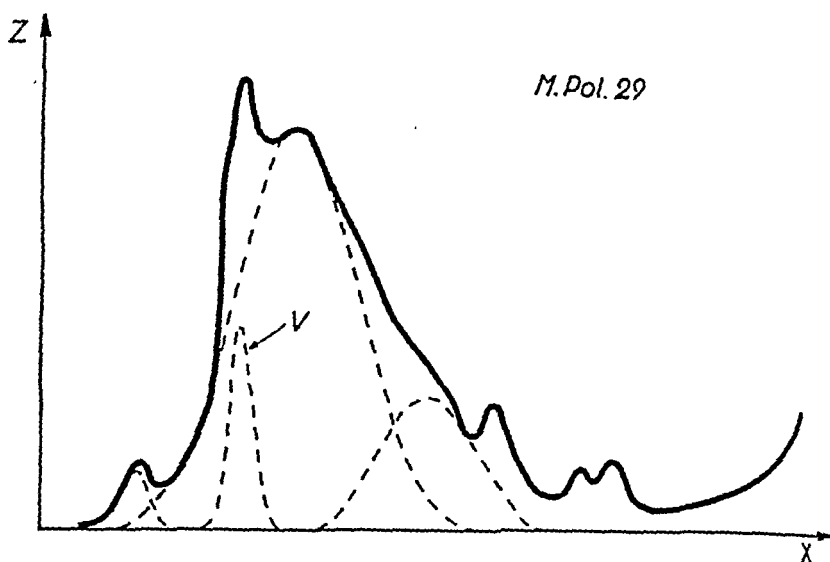


Figure 10. Sedimentation diagram of preparation III.  $V$  = virus component. Centrifugal field 32 000 g. Centrifugation time 10 minutes.

the others inhomogeneous. The most distinct of these has the constant  $195 \times 10^{-13}$ , the third is still higher. Fig. 11 demonstrates the sedimentation of preparation V, purified in the same way as preparation III. The homogeneous component has the constant  $153 \times 10^{-13}$ .

Figure 12 contains diagrams obtained with two different preparations. At the bottom are two exposures from the same run of preparation VII (experiment 21), showing three distinct components with sedimentation constants 39, 165, and  $232 \times 10^{-13}$ . The top diagrams are the corresponding exposures of a preparation of normal

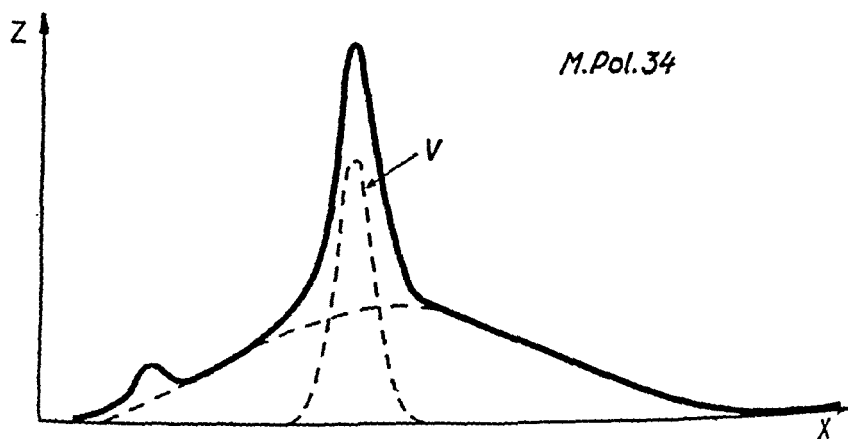


Figure 11. Sedimentation diagram of preparation V.  $V$  = virus component. Centrifugal field 32 000 g. Centrifugation time 17 minutes.

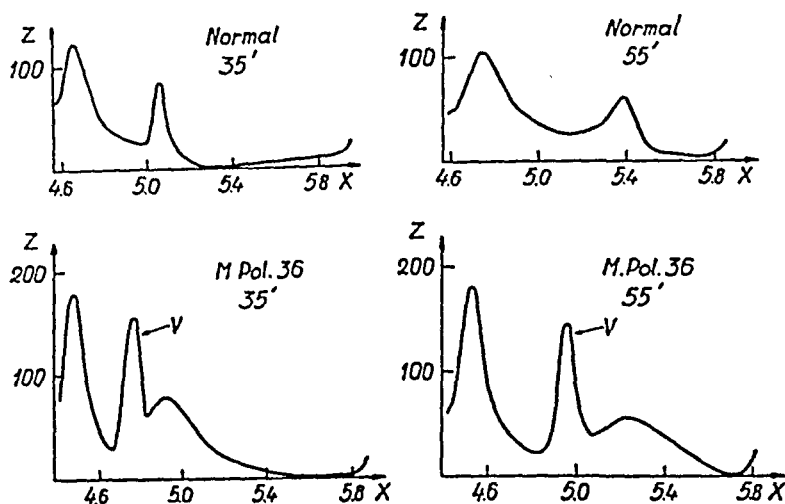


Figure 12. Sedimentation diagrams of purified normal material (top) and virus containing brain (bottom). V = virus component. Centrifugal field 18 800 g. Centrifugation times 35 and 55 minutes.

brains, purified in exactly the same way. Only two components are visible, the constants of which are 40 and  $229 \times 10^{-13}$ . Thus material from infected and from normal brains had two components in common, while the infected brain alone contained a third and very distinct one, the sedimentation velocity of which was of the same order of magnitude as that of the virus.

Figure 13 shows the sedimentation of preparation IX (experiment 23). In this case the substance was apparently homogeneous. No

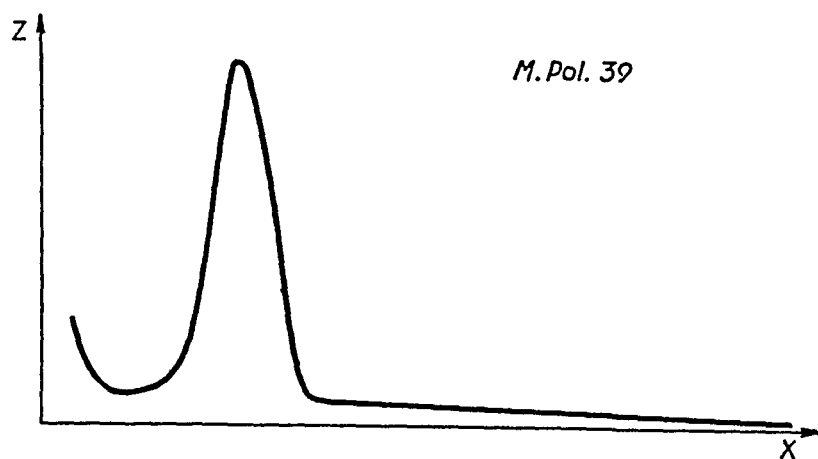


Figure 13. Sedimentation diagram of preparation IX. Homogeneous. Centrifugal field 18 800 g. Centrifugation time 31 minutes.



traces of impurities could be detected. The sedimentation constant was  $181 \times 10^{-13}$ . Unlike the previous centrifugations, in which distilled water was used as medium, this one was carried out in phosphate buffer of ionic strength  $\mu = 0.05$ . When the experiment was repeated later, using distilled water, a sedimentation constant of  $159 \times 10^{-13}$  was obtained. Experiment 23 was, however, exceptional. In three more cases, when the same purification method was employed, sedimentation diagrams like figure 14 were recorded. The characteristic feature was that the meniscus side of the gradient was normal, whereas the bottom side was disturbed. In later exposures of the same run the inhomogeneity would often have spread completely,

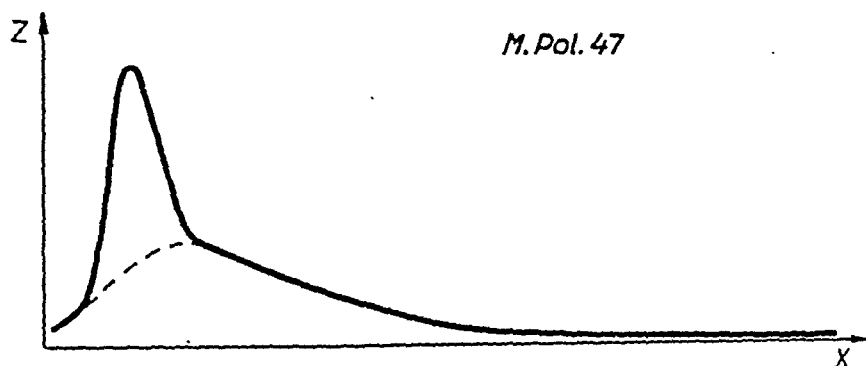


Figure 14. Sedimentation diagram of purified virus showing disturbance of the gradient. Centrifugal field 18 800 g. Centrifugation time 22 minutes.

so that no gradient referable to a disturbing component could be discovered. The preparation would then appear homogeneous. The inhomogeneous substance amounted to from 20 to 50 p. c. of the total contents of solids. For reasons to be discussed later it was assumed that this component consisted largely of the homogeneous substance in aggregated state and with adhering particulate impurities.

In the experiments now described it was found that purified and concentrated material from infected brains contained a substance that was not present in normal brains similarly treated. This substance displayed homogeneity of sedimentation with a sedimentation constant approximately equal to that of the virus as determined by means of biological recording. It was distinguishable in all preparations with a titer above  $\log A = 9.50$ . When the concentration was calculated from the sedimentation diagrams and compared with the titer of the corresponding preparation the following series was obtained (table XXII).

Table XXII.

Connection between concentration of virus protein and titer.

In the calculation of concentration the refractive index was assumed arbitrarily to be that of proteins.

Preparation	Concentration of virus protein p.c.	Titer	MID per mg
IV.....	Not distinguishable	8.81	—
II.....	Uncertain, below 0.010 p.c.	9.30	More than $2.0 \times 10^{10}$
VI.....	0.010 "	9.70	$5.0 \times 10^{10}$
III.....	0.015 "	10.21	10.7
XXI.....	0.021 "	10.25	8.5
V.....	0.025 "	10.24	6.9
VII.....	0.075 "	10.34	3.0

There is an unmistakable correspondence between the two entities. The last column contains the number of MID per mg of substance. These figures show a variation that might appear systematic but is probably merely incidental. Firstly it is of the same order of magnitude as the standard error of titration, secondly there is apparently no connection whatsoever with purification methods employed or degree of purity of the preparations. It might therefore be assumed that the activity is proportional to the concentration of the component in question.

All those circumstances taken together strongly suggest that *the substance now discussed represents the virus protein itself*.

So far no attempts have been made to analyze it chemically, as the only apparently pure preparation obtained (prep. IX), amounting to a total of 0.6 mg, was used for determination of the diffusion constant. During the sedimentation studies, however, some observations of a certain interest from a physico-chemical point of view have been made.

In 7 runs on 4 different, comparatively impure preparations (less than 15 p.c. virus), the sedimentation constant was calculated to  $150 \pm 4.3 \times 10^{-13}$ . 6 highly purified preparations (25—100 p.c. virus) gave in 7 runs an average of  $162 \pm 6.1 \times 10^{-13}$ . The difference,  $12 \pm 7.5$  is not statistically significant but indicates strongly that aggregation occurs during the purification process. We will return later to this question.

As already mentioned, preparation IX had a sedimentation constant of  $181 \times 10^{-13}$  in dilute buffer solution as compared with  $159 \times 10^{-13}$  in distilled water. The difference is too big to be incidental. The signification of this fact is not immediately obvious. It might be interpreted as a salt effect on the state of hydration, similar to that studied by Bernal and Fankuchen (14) in tobacco mosaic virus (cf. page 147).

The yield of virus was surprisingly uniform. In 7 experiments it was 0.43, 0.83, 0.50, 1.67, 0.55, 0.21, and 0.50, averaging 0.60  $\gamma$  per mouse brain and representing from 20 to 50 p. c. of the original virus content. This is in close agreement with the biological experience. As already mentioned, the virus multiplies in the CNS until a certain critical level is attained. This level seems to be never exceeded but always reached, and appears to be a characteristic of the strain of virus.

## II. Human neurovirus.

The scarcity of monkeys made it impossible to control in detail the applicability on the human virus of the experience gained in the purification experiments of the preceding section. It was therefore decided to apply the final method of purification to human material and to study the preparations in the ultracentrifuge, in order to ascertain, whether or not any components comparable to the mouse virus protein could be obtained.

The preparation of extracts, treatment with ether, precipitation with ammonium sulphate (40 p. c. saturation), and ultrafiltration was carried out as previously described. The concentrate was fractionated twice in the Beam's centrifuge (run up-and-down to 16 000 r. p. m. and 70 minutes at 27 000 r. p. m.). The final sediment was resuspended in 1 ml of distilled water.

In a preliminary experiment a normal control from two brains and spinal cords was prepared. When analysed in the ultracentrifuge it proved to contain one single, comparatively homogeneous component, the sedimentation constant of which was about  $60 \times 10^{-13}$ .

Next a material consisting of brains and spinal cords from two cases of poliomyelitis was purified (preparation XXX). In the ultracentrifuge the normal component was observed and besides that traces of a faster sedimenting substance. The sedimentation constant of the latter was probably below  $200 \times 10^{-13}$  but could not be

determined with any degree of accuracy on account of the low concentration. 0.05 ml of this material was inoculated intracerebrally to a *Cynomolgus* monkey, MC 1. The temperature chart of this animal is shown in fig. 15. A rise in temperature occurred on the 9th day after inoculation, followed by a collapse-like fall on the 12th day. On the 11th day the first motor symptoms were evident, consisting of paresis of the left arm. On the 12th day complete paralysis of the left arm and the right leg, prostration. The animal was sacrificed. No gross lesions were found at autopsy. Sections of pons and spinal cord showed typical lesions (Plate II). It was thus obvious that

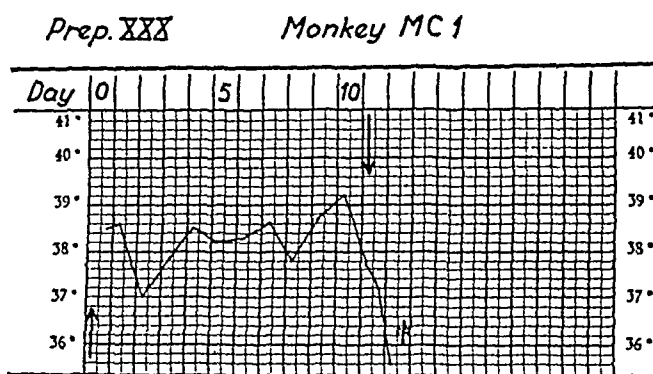


Figure 15. Temperature chart of monkey MC 1. ↑ time of inoculation. ↓ onset of paralysis. × animal sacrificed.

preparation XXX contained virus, although the concentration was too low for optical recording. Titration of the activity was unfortunately out of question.

Finally a pool of material from 11 cases was purified. This time the pedunculi cerebri et cerebelli were cut through and cerebrum and cerebellum discarded. The remainder — midbrain, pons and medulla oblongata et spinalis — amounted to about 5 p. c. of the total CNS, while its virus content can be estimated to about 90 p. c. of the total. By this operation, therefore, a substantial simplification of the purification procedure should be attained without any considerable loss of virus. The final preparation (XLVII) was faintly yellowish red in colour like the two preceding ones, but appeared in contrast to these to be inhomogeneous. On high speed centrifugation a small amount of an oily sediment was deposited, as in the earlier preparations, but in addition a minute, clear, gelatinous pellet was

present, of the same general appearance as the preparations of the mouse virus. The sedimentation diagram showed the presence of the normal component, and furthermore two additional homogeneous components in low concentration were distinguishable (fig. 16). The sedimentation constants of these were about  $100 \times 10^{-13}$  and  $150 \times 10^{-13}$ . The latter value is of the same order of magnitude as that of

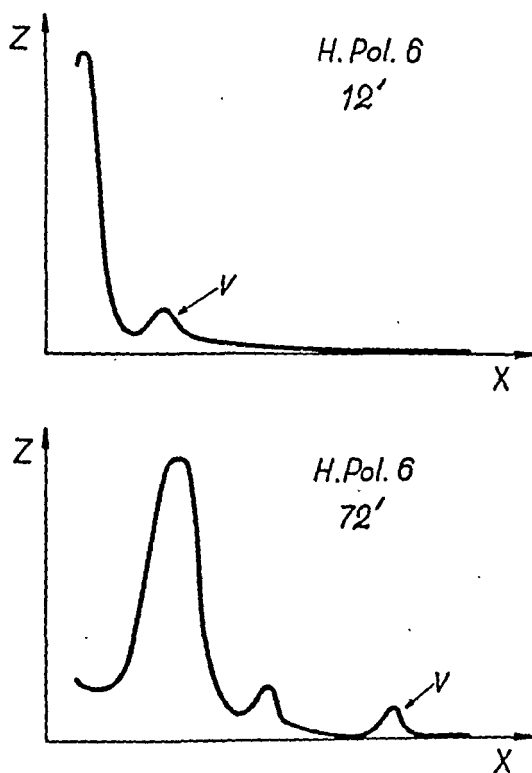


Figure 16. Sedimentation diagram of preparation XLVII, human poliomyelitis. *V* = virus component. Centrifugal field 18 800 g. Centrifugation times 12 and 72 minutes.

the mouse virus. Activity tests on preparation XLVII could not be performed.

The results of these experiments on the human neurovirus were less conclusive than those on the mouse virus. Firstly activity tests could not be carried out to the desirable extent, secondly insufficient amounts of material were prepared. Thus it has not been ascertained, whether one or both of the additional components in the last experiment are not normal constituents of the spinal cord, as the control was not directly comparable to this preparation. It might, however, be assumed that the component, which was traced in preparation

XXX but absent in the control, is identical with the fastest sedimenting substance in preparation XLVII, and that the presence of this substance is referable to the fact that the preparations were derived from infected material. As one of the preparations was shown to be active, it must be considered plausible to assume that the activity is connected with the component in question. At least a fraction of the virus, therefore, seems to have a sedimentation constant of about  $150 \times 10^{-13}$ , i.e. within the limits of the experiment the same as that of the mouse virus. The question whether or not the middle component of preparation XLVII has any connection with the activity can not be settled at present.

## CHAPTER VI.

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### Purification of intestinal virus.

With the exception of the experiments by the present writer already mentioned (46), no attempts to purify virus from stools or intestinal contents have been reported.

The general procedure adopted in the following experiments was a direct application of the experience from the purification of neurovirus. It was in detail as follows.

The material was suspended in a suitable volume of 0.3 p. c. saline solution and shaken with glass beads in a shaking machine for 2—4 hours. Further diluent was then added and the mass extracted under mechanical stirring for 8 hours. It was then left to settle in the cold room over night. The volume of fluid added was chosen so as to let the sediment amount to 10—15 p. c. of the total. The supernatant was siphoned off and passed twice through the separator. The clarified extract was mixed with 2/3 by volume of saturated ammonium sulphate solution and 1/10 by volume of ether, thoroughly shaken and left to settle in a separatory flask. After 4—8 hours the clear bottom layer was drawn off and the top layer resuspended in 3 times its volume of distilled water with an excess of ether. After 12 hours in the cold room the extract was collected and the top layer extracted further 2 or 3 times. The pooled extracts were clarified in the separator and concentrated through ultrafiltration. The concentrate, usually dark brown, turbid, and viscous, was run in the preparatory centrifuge at 27 000 r. p. m. for 90 minutes. The supernatant was discarded and the sediment allowed to swell in distilled water for at least 2 hours. It was then resuspended to the original volume in distilled water and run up-and-down to 16 000 r. p. m. The second supernatant was further fractionated in the centrifuge, alternatingly at 27 000 r. p. m. for 70 minutes and up-and-down to 16 000 r. p. m.

Most of the following work was done on material of human origin. In order to avoid unnecessary reiterations these experiments will, therefore, be described first.

## I. Experiments on human stools.

### *Differential centrifugation.*

A pool of 6 samples from cases of poliomyelitis was prepared as described above. The purified extract was brown in colour, almost clear but with a strong Tyndall cone. On centrifugation at 27 000 r. p. m. for 70 minutes a brown translucent gelatinous pellet was deposited. This pellet was stratified in structure, probably an indication of inhomogeneity. It was soluble in distilled water, more easily after swelling in a small volume of water for 1 or 2 hours. When analysed in the ultracentrifuge it showed the presence of three main components with sedimentation constants 147, 210, and  $276 \times 10^{-13}$ . The solution = preparation c was subjected to further purification as follows.

The volume was made up with distilled water to 100 ml, giving a very faint colour but a distinct Tyndall phenomenon. The solution was run up-and-down to 22 000 r. p. m. The supernatant was collected, the sediment redissolved in 100 ml of distilled water and again run up-and-down to 22 000 r. p. m. The procedure was repeated until the supernatant was free of slower sedimenting material, in all 5 times. The final sediment was redissolved in distilled water = fraction  $\gamma$ .

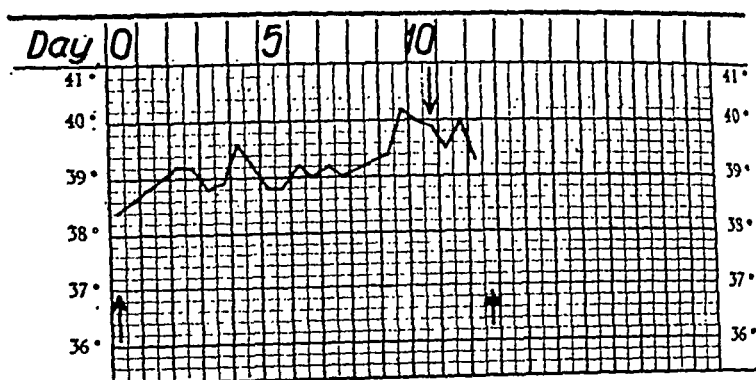
The pooled supernatants were concentrated through ultrafiltration to a volume of 100 ml = preparation b, and fractionated in the centrifuge in principally the same way as before, only being run up-and-down to 27 000 r. p. m. instead of 22 000. More than 20 runs were necessary to make the separation complete. The final sediment was redissolved in distilled water = fraction  $\beta$ .

The second pooled supernatants were again concentrated through ultrafiltration to a volume of 100 ml = preparation a. The solution was centrifuged at 27 000 r. p. m. for 25 minutes. As a complete sedimentation of the material seemed to occur no further fractionation was attempted. The sediment was redissolved in distilled water = fraction  $\alpha$ . In each step of this fractionation procedure the centrifugal effect applied was approximately twice that of the preceding one.

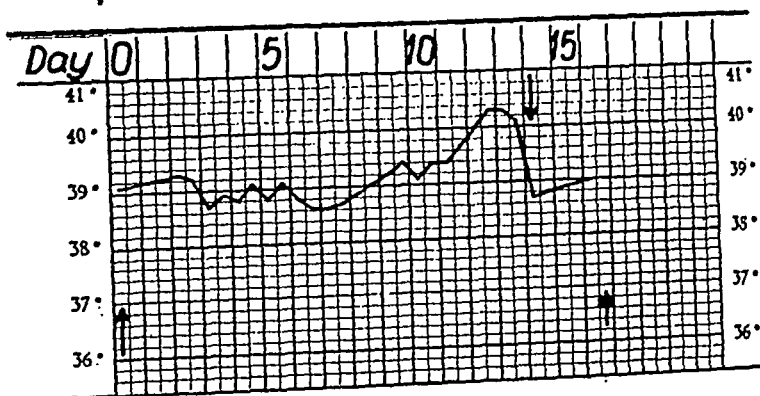
Thus preparation c contained fractions  $\alpha$ ,  $\beta$ , and  $\gamma$ , preparation b fractions  $\alpha$  and  $\beta$ , and preparation a only fraction  $\alpha$ . Of each solution 0.5 ml amounts had been inoculated intracerebrally into monkeys. All three animals came down with typical poliomyelitis. Their temperature charts are shown in fig. 17 and micrographs of sections from the CNS in plate II.



*Prep. XXII c Monkey MR 5*



*Prep. XXII b Monkey MR 6*



*Prep. XXII a Monkey MR 8*

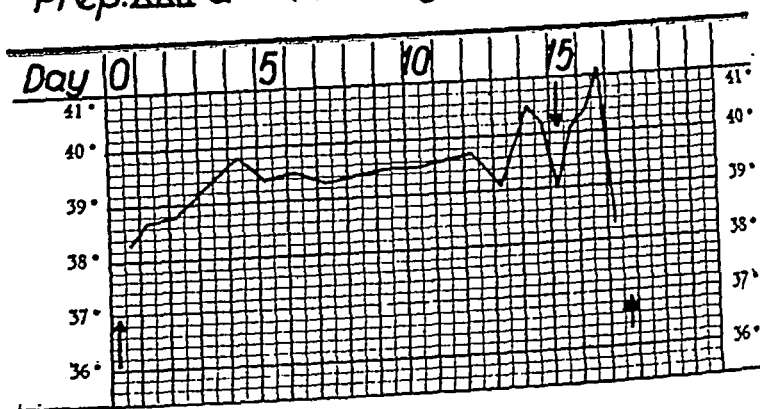


Figure 17. Temperature charts of monkeys MR 5, 6, and 8. ↑ time of inoculation. ↓ onset of paralysis. + animals sacrificed.

*Rhesus monkey MR 5*, preparation *c*: Rise in temperature on the 9th day. Paresis of left arm on the 10th day. On the 12th day temperature was back to normal, the paresis slightly more pronounced but no further motor symptoms had appeared.

*Rhesus monkey MR 6*, preparation *b*: Rise in temperature on the 11th day, reaching a peak on the 12th and 13th day, back to normal on the 14th day, when paresis of the hind quarter was observed. On the 15th day complete paralysis of legs, paresis of left arm. General condition good. 16th day no further development of symptoms.

*Rhesus monkey MR 8*, preparation *a*: Rise in temperature on the 14th day. Remission to normal on the morning of the 15th day, paresis of right leg. New rise in temperature in the afternoon, culminating on the following evening, when complete paralysis of hind quarter and paresis of left arm were observed. On the morning of the 17th day temperature below normal. Animal prostrated.

Preparation *c* had a total volume of 12 ml, *b* and *a* 100 ml, as already mentioned. Monkey MR 5 therefore received an amount of fractions  $\alpha$  and  $\beta$  about ten times that administered to MR 6. The amounts of fraction  $\alpha$ , given to MR 6 and MR 8 were approximately equal. The fecal specimens were collected on October 14. Preparation *c* was ready for inoculation on November 8, preparation *b* on November 29, and preparation *a* not until January 28, 3 1/2 months after the start of the experiment.

A gradual increase of the incubation period was evident. On the other hand the clinical course of the experimental disease was gradually aggravated. When all circumstances are taken into consideration, the assumption that *the activity had principally followed fraction  $\alpha$*  seems best to meet with the facts.

The three fractions were very similar in appearance. They all formed brown, translucent pellets. The colour of  $\gamma$  was deep brown,  $\alpha$  and  $\beta$  were of a somewhat lighter shade.  $\alpha$  seemed to be more easily soluble than the others. The solutions were clear with a pronounced Tyndall phenomenon. When observed in the dark field (magnification  $600\times$ )  $\gamma$  contained a mass of particles, just about visible, often in larger aggregates, whereas no structures whatsoever could be detected in solutions of  $\alpha$  and  $\beta$ .

The solutions (in distilled water) were analysed in the ultracentrifuge. Each fraction consisted of one single component. The sedimentation constants were:  $\alpha = 42$ ,  $\beta = 79$ , and  $\gamma = 309 \times 10^{-13}$ . The diagrams of  $\alpha$  and  $\beta$  indicated that their sedimentation velocity was dependent upon their concentration. In a number of experiments

this dependence was studied. The result is summarized in the diagram in fig. 18, sedimentation constant on the ordinate, and concentration on the abscissa. The two curves, not referring to absolute concentrations, are comparable only concerning their general shape. Dry weight determinations were not carried out and calculations of the concentration from the sedimentation diagrams were at least uncertain, since the nature of the substances and their refractive

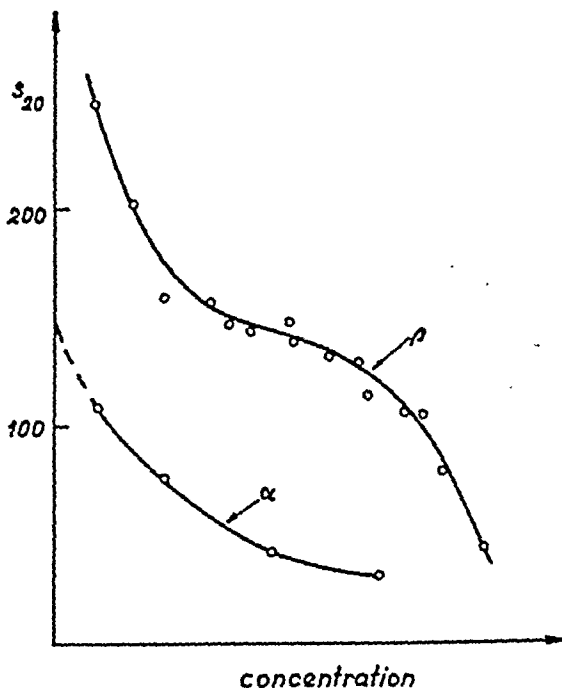


Figure 18. Sedimentation velocity-concentration curves of the  $\alpha$ - and  $\beta$ - component of human stools.

indices were unknown. From fig. 18 it is evident, that under certain conditions separation of a mixture through centrifugation might be impossible, and that the two components might sometimes be made to change places in the sedimentation diagram by adjustment of the concentration. If, however, the mixture is diluted above a certain limit the difference in the rates of sedimentation can always be brought to an amount that makes separation although laborious yet possible. Hence the dilution to 100 ml in the above separation procedure.

For many substances showing variation of sedimentation velocity with concentration the following formula is approximately valid

$$s_c = s_0 \cdot \frac{1}{1 + kc}$$

where  $s_c$  and  $s_0$  denote the sedimentation constants at the concentrations  $c$  and 0, and  $k$  is a constant. In those cases  $s$  plotted against  $c$ ,  $s$  will give a straight line. The use of the latter method of plotting often simplifies extrapolation of the value of  $s_0$ . As the  $\alpha$  component seemed to follow this formula, the ultimate value of its sedimentation constant for the concentration 0 could be estimated to about  $165 \times 10^{-13}$ . On the  $\beta$  component on the other hand the formula was not applicable, and in estimating the upper limit of the sedimentation constant great caution was necessary. The value was probably above  $300 \times 10^{-13}$ .

#### *Effect of sodium chloride.*

The presence of small amounts of salt had a very marked effect on the sedimentation. The  $\gamma$  component seemed to be practically insoluble even in 0.01 m sodium chloride solution and could easily be disposed of through centrifugation at 3000 r. p. m.  $\beta$  appeared more homogeneous in salt solutions than in distilled water (fig. 19). The rate of sedimentation was higher and less dependent upon the concentration. The upper limit appeared to be about  $280 \times 10^{-13}$ . The salt effect on the  $\alpha$  component was somewhat less pronounced. The upper limit of  $s_{20}$  was probably about  $220 \times 10^{-13}$ , and values below  $100 \times 10^{-13}$  were exceptional.

It was observed that the pellets deposited at high speed centrifugation from saline solutions were smaller in volume than those obtained from the same preparation in distilled water. They were also less readily soluble. It was probable, therefore, that the salt effect consisted mainly of a dehydration of the substances. As a hint that very high degrees of hydration sometimes were attained, the occurrence of sedimentation velocity curves of the type shown in fig. 20 was considered. The gradually decreasing rate of sedimentation was very conspicuous. Such curves were observed only when distilled water was used as solvent, and most often only the  $\beta$  component was involved. On some occasions though, as in the example quoted, pure  $\alpha$  solutions showed this phenomenon as well. This is the type of curve one would expect in a gel, where most of

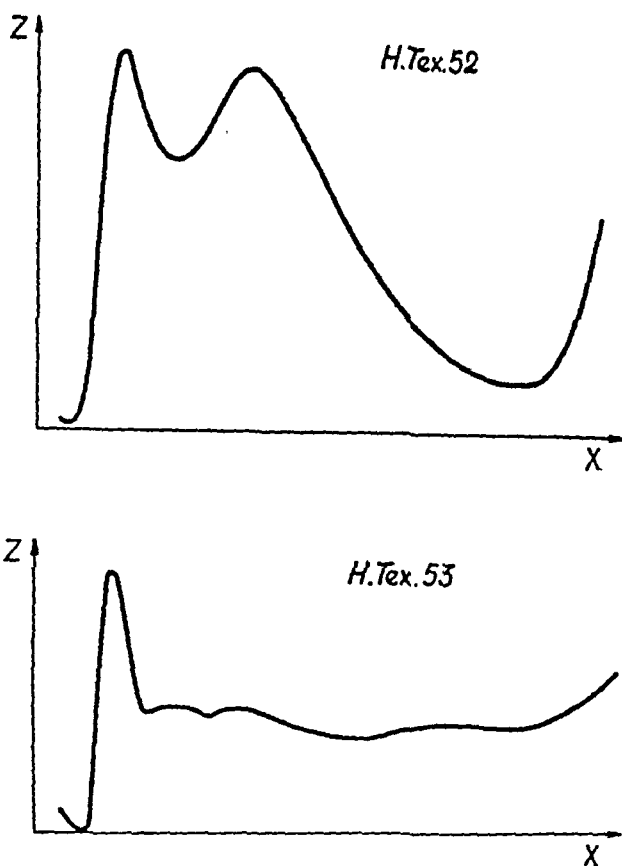


Figure 19. Sedimentation diagram of the same preparation in 0.05 m sodium chloride (top) and distilled water (bottom). The  $\beta$ -component inhomogeneous in absence of salt. Centrifugal field 18 800 g. Centrifugation times 41 and 120 minutes.

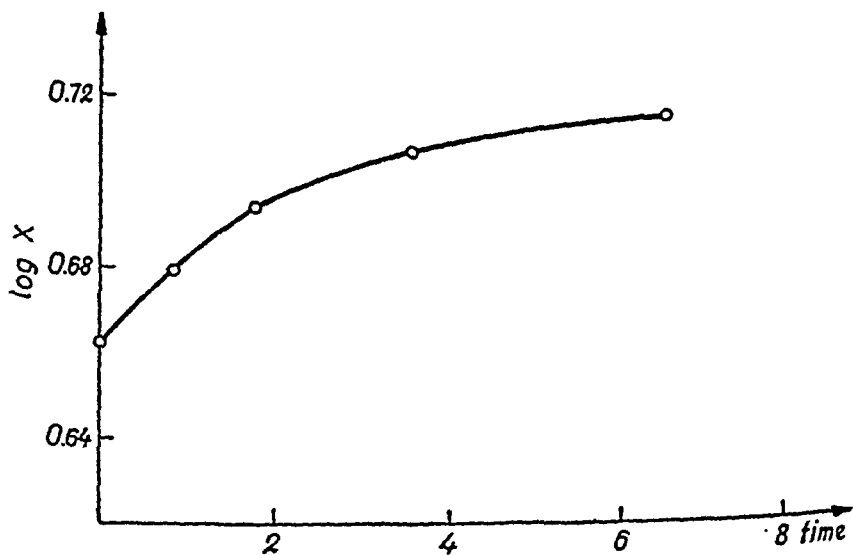


Figure 20. Gellike sedimentation of the  $\alpha$ -component in distilled water. Rate of sedimentation gradually decreasing.

the water is fixed. The sedimentation would, in fact, be expected to meet with an increasing resistance, as the force necessary for dehydration increases with the amount of water pressed out of the gel. The sedimentation of gels, however, is not yet sufficiently studied, so the interpretation of the phenomenon now described remains undecided.

In solutions, containing more than one component, conditions were sometimes very complicated. As an illustration fig. 21 shows

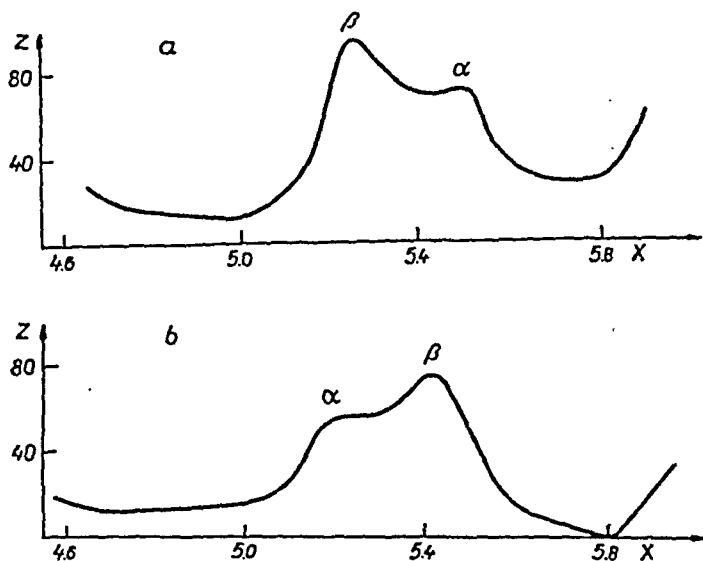


Figure 21. Sedimentation diagrams of the same preparation in distilled water (top) and 0.05 m sodium chloride (bottom). Centrifugal field 18 800 g. Centrifugation times 100 and 72 minutes.

two sedimentation diagrams of the same preparation. In distilled water the  $\alpha$  component sediments faster, in 0.3 p. c. NaCl solution the  $\beta$  component. Still other irregularities seemed to be caused by the presence of further impurities. On the whole it was found impossible to identify the components of a preparation by sedimentation analysis alone, and the hope that this procedure might be available for diagnostic purposes had to be abandoned.

The presence of small amounts of sodium chloride seemed, however, in a way to stabilize the conditions of the solution and make the state of the system more well-defined. It should therefore be preferable to carry out the differential centrifugations using a saline solution as solvent. The standard diluent, 0.3 p. c. NaCl solution, was tried

for this purpose and found to yield quicker results. A complete separation of  $\alpha$  and  $\beta$  was usually attained after 5 or 6 fractionations.

Solutions of the pure  $\alpha$  component, obtained according to this method, gave sedimentation diagrams like fig. 22 showing a disturbance of the sedimentation boundary of the same type as that observed in neurovirus (cf. fig. 14). This disturbance could not be eliminated by differential centrifugation regardless of the number of fractionations applied to the material.

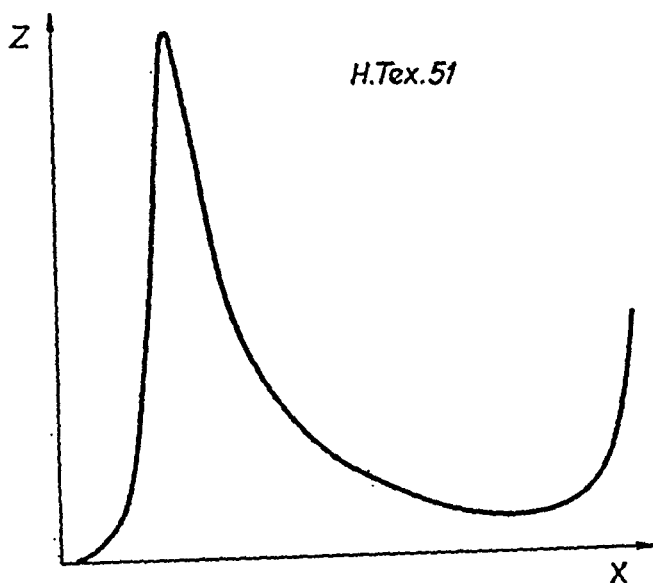


Figure 22. Sedimentation diagram of the  $\alpha$ -component showing disturbance of the gradient. Centrifugal field 18 800 g. Centrifugation time 41 minutes.

#### *Treatment with dilute ammonium sulphate solutions.*

In a series of experiments mixtures of  $\alpha$  and  $\beta$  had been centrifuged in glycerin solutions. The idea was to use differences in specific gravity for separation purposes. Table XXIII illustrates one such experiment. The constants tabulated are not corrected to the density and viscosity of water. The experiment showed that no measurable divergence of the rates of sedimentation was attained by increasing the density of the solvent. The densities of the two substances must therefore be nearly equal and furthermore well above 1.07, approximate specific gravity of 30 p. c. glycerin solution.

For the same purpose ammonium sulphate solutions were tested. In these experiments it was observed that the sedimentation diagrams of solutions of the  $\alpha$  component were modified. Fig. 23 shows the same preparation when sedimenting in a dilute phosphate buffer

Table XXIII.  
Sedimentation velocities in glycerin solutions.

Solvent	$s_{20} \times 10^{13}$	
0.3 p. c. NaCl .....	167	197
» + 10 p. c. glycerin.....	115	155
» + 20 p. c. » .....	65	94
» + 30 p. c. » .....	51	70

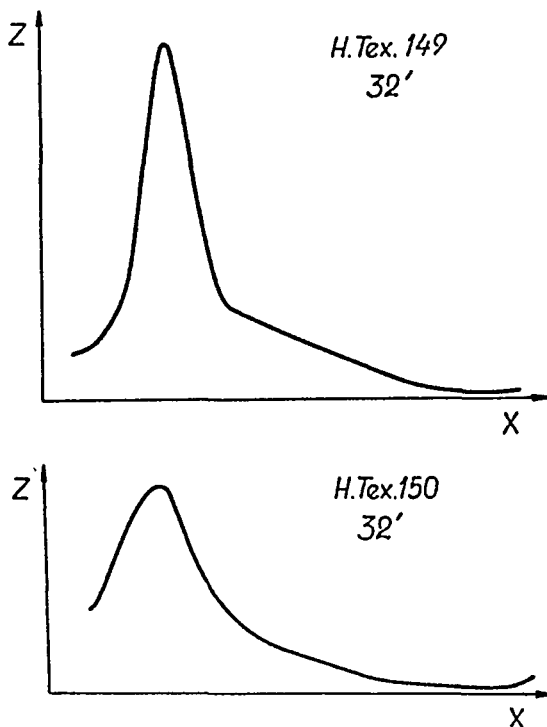


Figure 23. Sedimentation diagrams of the  $\alpha$ -component in 0.05 m sodium chloride (top) and 0.2 m ammonium sulphate (bottom). Centrifugal field 18 800 g. Centrifugation times 32 minutes.

solution  $\mu = 0.01$  (top) and in 0.2 m ammonium sulphate (bottom). Compared with the control, the bottom diagram showed a higher degree of polydispersity and the disturbance of the gradient was most pronounced on the meniscus side instead of the bottom side. A similar alteration was not observed in 0.2 m NaCl solution or in glycerin medium, neither did it occur in solutions of the  $\beta$  component.



As an attempt to explain this observation it was assumed that the disturbance of the gradient was due to low molecular impurities, firmly adsorbed on the conjectured virus protein. The ammonium sulphate solution should act as an eluent. The free low molecular material would sediment slower, causing a disturbance of the meniscus side of the gradient, while the virus protein, deprived of its protecting coating would be liable to aggregate with polydispersity as consequence.

If this interpretation were correct it should be possible to improve the purity of the material by treatment with ammonium sulphate solutions of a suitable concentration. The elution of the impurities could be expected to be a reversible process, so they should be removed, while the salt concentration was maintained at the most favourable level. To judge from the diagrams, differential centrifugation did not appear to be an ideal method for separation. Instead of that etherization was tried.

To 45 ml of purified  $\alpha$  solution 5 ml of saturated ammonium sulphate solution and 10 ml of ether were added, the mixture vigorously shaken in a separatory funnel and left to separate. Beneath the ethereal layer a distinct brown pellicle had formed. The aqueous bottom layer was drawn off and, after removal of the ether, fractionated in the centrifuge. Hereby a further amount of insoluble material could be removed through low speed centrifugation.

The final preparation was almost colourless with a distinct bluish Tyndall cone. When analysed in the ultracentrifuge, it appeared to be more polydisperse than the original preparation, although the previous disturbance of the gradient was not distinguishable (cf. fig. 24).

### Occurrence of the $\alpha$ component.

In October 1941 the first series comprising a total of 42 specimens from 6 cases of poliomyelitis was examined. The material was collected at intervals of 2 or 3 days and pooled as indicated in table XXIV. The average time between onset of symptoms and the collection of the specimens included in the different pools varied from 12 to 29 days. Each pool was prepared separately. From all of them the  $\alpha$  component could be isolated. The yield was roughly proportionate to the number of specimens included. There was definitely no decrease in amount with increasing length of time between onset of the disease and the date of the specimens.

Table XXIV.

Preparation of stool specimens from cases of poliomyelitis.

Patient .....	B. K.	O. W.	W. B.	I. B.	I. M.	B. W.	Pool No.	Average time after onset
Onset of disease	26. IX	1. X	1. X	2. X	3. X	8. X		
Specimen collected:								
14. X .....	+	+	+	+	+	+	XXII	12 days
16. X .....	+	+	+	+	+	+	XXIII	14 "
18. X .....	+	+	+	+	+	+	XXIV	16 "
20. X .....	+	+	+	+	+	+	XXV	18 "
22. X .....	+	+	+		+	+	XXVI	20 "
24. X .....	+	+	+	+	+	+	XXVII	22 "
26. X .....	+	+	+		+	+	XXVIII	24 "
29. X .....				+			XXIX	20 days
2. XI .....				+				

In a following series a total of 16 specimens were prepared individually. Of these 6 were collected within one week, 4 in the second week, 2 in the third week, one 3 months, one 4 1/2 months, one 6 months, and one 17 years after onset of the disease. All specimens contained the  $\alpha$  component in approximately the same amount, 0.1—1 mg per specimen.

Finally 106 specimens from 27 different patients were examined, partly in pools of 2—6 specimens. The  $\alpha$  component was consistently recovered. The yield varied with the amount of solids present in the specimen. It could be estimated to approximately 0.5 mg per 100 g of feces.

A series of controls included 12 specimens from healthy contacts, 2 from obscure lethal cases of polyneuritis, one from a case of acute encephalitis and 19 from miscellaneous cases such as neurosis, polyarthritis, diabetes, vitium cordis etc. 14 specimens were collected in February and March, when no cases of poliomyelitis had occurred within three months before, and the remainder in September during a small epidemic of poliomyelitis. From all of them a substance with the characteristics of the  $\alpha$  component was recovered to an amount comparable to that obtained from cases of poliomyelitis.

The experiments reported in this section had shown that a certain substance, here called the  $\alpha$  component, characterized by its precipitability with ammonium sulphate and its behaviour in a centrifugal field, is regularly present in human stools. As far as these experiments go it shows a striking resemblance to the presumed neurovirus protein found in brain and spinal cord from cases of murine as well as human poliomyelitis. Although activity tests could not be performed to the desired extent, suggestive evidence has been presented that in the process of purification most of the original amount of virus, present in active specimens, is to be found in the same fraction as the  $\alpha$  component. In such active preparations no further component has been detected that could account for the difference between active and inactive specimens.

## II. Experiments on mouse feces.

The material was collected as described in chapter II. Batches of about 100 g were prepared at a time. The general procedure of purification outlined in the preceding section was strictly followed.

The preparations thus obtained closely resembled those from human stools. On high speed centrifugation they deposited yellowish brown, gelatinous pellets. The solutions were light brown and clear, with a Tyndall cone. In centrifugal analysis usually only one component was observed. From a physico-chemical point of view this substance was identical with the  $\alpha$  component of human stools. The dependence of the rate of sedimentation upon the concentration was of the same order of magnitude. The upper limit of the sedimentation constant was about  $160 \times 10^{-13}$  in distilled water,  $190-200 \times 10^{-13}$  in 0.3 p. c. sodium chloride solution.

Sometimes a second component was present but always in comparatively small quantities. It was very sensitive to the presence of salt and therefore more like the  $\gamma$  than the  $\beta$  component.

The sedimentation diagrams of the main component showed a certain disturbance of the bottom side of the gradient, although as a rule to a less extent than the corresponding preparations from human stools. Through treatment with 10 p. c. ammonium sulphate and ether a small amount of impurities could be removed. A sedi-

mentation diagram of a preparation subjected to such additional purification was presented in fig. 24.

The material originated partly from young mice, 5—8 weeks of age, partly from 12—18 months old animals. These two types of preparations showed no differences with regard to physico-chemical properties or yield. The amount of the substance excreted was estimated to about 0.01 mg per mouse and day.

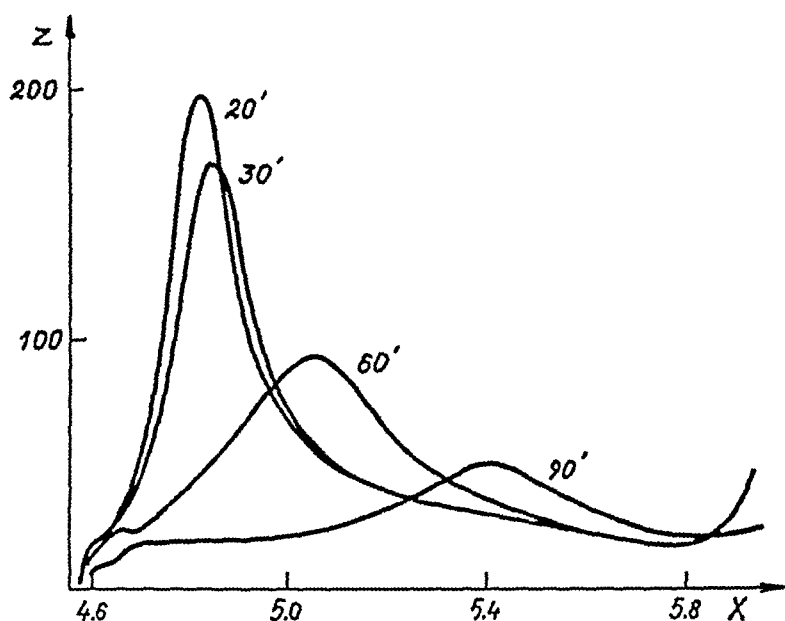


Figure 24. Sedimentation diagrams of mouse intestinal protein, showing polydispersity. Centrifugal field 18 800 g. Centrifugation times 20, 30, 60, and 90 minutes.

### Activity tests.

The strains of virus that can be isolated from the intestines of normal mice are usually of a very moderate virulence. In chapter III it was pointed out that titration of less virulent strains met with considerable difficulties. The incubation time method is not applicable. The variation of individual resistance plays an important part in a titration experiment, making the series irregular and the evaluation of the result hazardous.

A phenomenon called interference was reported now and again by workers in the virus field. It was observed that avirulent strains of certain viruses, when inoculated simultaneously with highly

virulent strains of the same virus, modified the course of the ensuing disease or sometimes inhibited it completely. Interference is usually interpreted as a competition for the susceptible cells, this interpretation being based upon the assumption, that a cell once invaded by a virus particle is refractory to superinfection by the same virus. Jungeblut and Sanders (78) have reported experiments with a murine strain of poliomyelitis virus supposed to interfere with simian strains. Similar observations have been made by the present author (unpublished data) on intestinal strains of the mouse poliomyelitis virus and the FA strain. The experiments in question were carried out with impure neurovirus. It was decided to extend this investigation to purified preparations in an attempt to use the degree of interference as a measure of the concentration of the interfering virus.

*Experiment 24.* In this experiment a purified preparation of feces from young animals was used (preparation XXXVI). It was diluted to 1: 50 and from this standard solution a series of 10fold dilutions was prepared. An extract of infected brains (strain FA) was similarly diluted, starting with 1: 500 (brain wet weight). The dilutions of the feces preparation were mixed with equal volumes of each one of the FA dilutions. Four dilutions of each solution were used making a total of 16 mixtures. Each mixture was inoculated intracerebrally in 0.02 ml amounts to groups of 20 mice. Control titrations of each one of the two solutions were also included. The activity of each mixture was calculated from the incubation time in the usual way and compared with that of the control containing the same concentration of strain FA. The differences  $= \log A - \log A_0$  are tabulated in table XXV.

Table XXV.

Effect upon the activity of the FA strain by addition of purified intestinal virus.

For explanation of the table see text.

Concentration of FA	Concentration of preparation XXXVI			
	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$
$10^{-3}$ .....	+ 0.65	+ 0.33	- 0.35	+ 0.07
$10^{-4}$ .....	+ 1.64	+ 0.02	+ 0.04	+ 0.18
$10^{-5}$ .....	+ 2.33	+ 1.07	+ 0.16	+ 0.11
$10^{-6}$ .....	+ 3.26	+ 2.05	+ 1.07	- 0.07

This table is divided into two sections by a heavy line. The values to the right of the line of division are not significantly different

cases was the exanthema immediately preceded by an acute infectious disease, in both cases an angina.

In a couple of cases E. m. appeared as *a link in a chain of tuberculous manifestations*. Example: A 20-year-old woman had first a tuberculous anal abscess, 8 months later erythema nodosum, 6 months after this a recurrence of the anal abscess, at the same time E. multiforme with some few E. nodosum nodules, pulmonary infiltration and vesiculous tuberculin reaction. Three years later fully developed cavernous phthisis.

There is reason to devote particular attention to *the result of the tuberculin test* in cases of E. multiforme. Occasionally there may be seen already in the first 24 hours typical E. multiforme efflorescences around the Pirquet scratch, with papules and vesicles. It is important to bear in mind that this does not necessarily imply a positive, specific reaction, since this phenomenon may also appear in cases where subsequent careful control tests with tuberculin yield negative results. The same typical efflorescences may be seen around a control scratch made without use of tuberculin. Sometimes a large bulla is found around the Pirquet scratch, and I have also seen bleeding therein in case of a tuberculin-negative patient with hemorrhagic E. m. Sure conclusions can in such cases be drawn from the tuberculin test only after the eruption has disappeared.

On rare occasions we find in patients with E. m. a generally increased cutaneous sensitivity, which finds expression in positive reaction to a number of specific and unspecific products, as Owren, for example, has shown in a case from Medical Dept. A of the Rikshospital. Such cases seem to represent exceptions. Rotnes in several of his cases made investigations as to the reaction to NaCl, milk, broth, serum, staphylococcal and streptococcal emulsions, trichophytin, and found no general increase in cutaneous sensitivity. Likewise Löfgren's results indicate that in the great majority of cases the reactions to tuberculin or to streptococcal emulsion are specific, and I have come to the same result in a large number of cases. Great cutaneous sensitivity is stated to be characteristic of *Behcet's syndrome*. According to T. Jensen, however, the sensitivity here differs somewhat from that mentioned above, seeing that there comes a pustule 24 hours after puncture with a needle in skin or mucous membrane, while no reaction is obtained by scarification of the epidermis. H. Rygh found no pronounced cutaneous sensitivity in his case of Behcet's syndrome.

### III. Experiments on further animal species.

Since it had been found that a substance, by the available methods of analysis undistinguishable from the  $\alpha$  component of human stools, also seemed to be a normal constituent of mouse feces, it became necessary to include into this study other species of animals as well. The preparation of the specimens was carried out exactly in the same way as before.

#### *Guinea pig.*

Minute amounts of substance were obtained, giving a greenish solution, faintly opalescent. In centrifugal analysis only slow sedimenting utterly inhomogeneous material was detected. No distinct components whatsoever were observed.

#### *White rat.*

When the preparation was carried out in distilled water comparatively large quantities of slow sedimenting inhomogeneous material were obtained. Most of this was insoluble in dilute sodium chloride solution. No distinct components could be detected in the ultracentrifuge.

#### *Swine.*

The intestinal contents of 3 pigs, a total of about 10 liters, were collected at slaughter, and purified as usual. The final preparation consisted of about 1 mg of a homogeneous substance, resembling the  $\alpha$  component with regard to sedimentation properties. Before definite conclusions be drawn, however, further studies are necessary.

#### *Wild brown rat (*Mus decumanus*).*

Wild rats were trapped on a garbage dump and caged. Their feces were collected and prepared in the usual way. The purified preparations contained consistently two homogeneous components in large amounts. One of those seemed to be identical with the  $\alpha$  component. The other had a lower rate of sedimentation and was only moderately sensitive to dilute salt solution. At repeated fractionation in the centrifuge it seemed to aggregate gradually and could be removed by and by through low speed centrifugation. After reprecipitation with ammonium sulphate (40 p. c. saturation)

was a case of Boeck's sarcoid. I have not found any record of the occurrence of E. multiforme in connection with that disease. Otherwise I may refer to another publication on this subject (Ustvedt: Further Observations concerning Bilateral Hilar Adenitis).

Thus we find that E. multiforme in the cases where tender nodules on the legs are to be found at the same time can probably be placed on a line with E. nodosum with respect to the importance of tuberculous primary infection as etiological factor. Further it is clear that also the unmixed E. multiforme without such E. nodosum efflorescences is sometimes, although far more seldom, due to tuberculous primary infection. On the other hand, it is doubtful whether tuberculosis plays any part whatever as causal factor in cases with manifestations from mucous membranes. An exception is here represented by *the cases with episcleritis*, which seem to stand in the same position as cases of combined E. m. and E. n. Where the exanthema is of bullous type the possibility of tuberculous etiology is evidently very small.

In this connection I may call to mind the difference which in the introductory description of the clinical picture was shown to exist between «the mucous membrane group» and «the combination group» (E. m. + E. n.). In the former group there was a certain preponderance of male patients, while in the latter 95 per cent were females, as in pure E. nodosum. The tendency to recurrence is mainly to be seen in the first group. On the other hand, the cases with combined E. m. and E. n. far more frequently presented prodromes in the form of dysphagia and joint-pains, such as are also seen in E. nodosum. Likewise the high values found for the sedimentation rate were to a certain degree characteristic of this group.

As regards the pure E. multiforme without mucous membrane symptoms and without E. nodosum efflorescences, which seems in every respect to occupy an intermediate position between the other two groups, it is not unreasonable to suppose that we have here to do partly with cases that are of the same kind as the combination cases, which is evidenced by, *inter alia*, their association with tuberculous primary infection, partly with cases of the same nature as the mucous membrane cases, as is indicated by, for instance, the appearance of bullous exanthema and tendency



man, white mice, and wild brown rats. A substance of presumably the same kind was also found in intestinal contents from swine, whereas no similar constituent was recovered from guineapigs nor from white rats. Judging from the present limited experience, therefore, this substance is not common to all species of animals.

With regard to sedimentation the  $\alpha$  component resembled closely the neurovirus protein. It was, therefore, quite natural that material derived from virus containing specimens seemed to have retained most of the viral activity originally present. When analysed in the ultracentrifuge, however, the active preparations differed in no way from the inactive ones. Thus, no component was detected, which the viral activity could be referred to in particular.

When purified intestinal virus from mice was added to the virulent FA strain and inoculated intracerebrally, no interference phenomenon appeared. The addition of intestinal virus enhanced the effect just as would an additional amount of the encephalitogenic strain itself. The  $\alpha$  component from non-infective mouse feces, in itself inactive even when injected in large quantities, had a similar although somewhat less pronounced effect when inoculated together with a virulent strain of virus. In one experiment a 50-fold increase in titer was observed.

As to the mechanism of this activation no conclusive evidence is available. An analogy to the spreading factor of Duran-Reynals (31) was considered. In a few experiments testis extracts were tested with negative result. Furthermore extracts of liver, spleen and kidney were examined. All of these had a slight although significant inhibitory effect, probably due to their content of serum. It is possible that an agent acting as spreading factor in nervous tissue should be expected to possess lipolytic rather than mucolytic properties. So far no experiments to that effect have been performed. The result of experiment 27 on rats gives a hint that the explanation of the phenomenon might be something in line with this suggestion.<sup>1</sup>

At any rate the presence in feces of a substance capable of enhancing the activity of poliomyelitis virus, together with the observation that infected nervous tissue contains an inhibitory factor (chapter V, page 97), explains satisfactorily the ease with which virus can be recovered from feces, and the difficulties to establish the strains in intracerebral passages.

<sup>1</sup> The problem will be reviewed later from another aspect. Cf. page 153.

## CHAPTER VII.

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### Further studies on purified material.

#### I. Physico-chemical studies.

##### Diffusion experiments.

From the filtration endpoint determinations the size of the virus of poliomyelitis was estimated to 10—15 m $\mu$ , or approximately the same as that of foot and mouth disease. Janssen (75) had found in centrifugation experiments with the latter virus a component with the sedimentation constant  $s_{20} = 20 \times 10^{-13}$ , interpreted by him as the virus protein.<sup>1</sup>

The sedimentation velocities recorded for the poliomyelitis virus were unexpectedly of a different order of magnitude. It was therefore essential to corroborate these findings by determination of the size and shape of the virus particles, based upon the sedimentation data. To this end diffusion experiments were carried out.

The technique employed was that described by Lamm (94). All experiments were carried out at 0° C and the figures corrected to + 20° C as usual. All preparations examined gave diffusion diagrams more or less of the type to be expected from polydisperse or inhomogeneous material. The constants calculated are, therefore, mean values the extreme limits being about  $\pm 10$  p. c. The following values were obtained (table XXVII).

For computation of the molecular weight the specific volume of the substance must be known. Proteins usually have a specific volume of about 0.75. For tobacco mosaic virus it was determined by Eriksson-Quensel and Svedberg to 0.646 (36).<sup>2</sup> This has not been confirmed by later workers. Values of about 0.77 have been recorded

<sup>1</sup> These experiments were carried out at the Institute of Physical Chemistry, Uppsala.

<sup>2</sup> The virus preparation available for these experiments was largely inhomogeneous. The possibility of a partial denaturation must be kept in mind.

Table XXVII.  
Diffusion constants.

	$D_{20} \times 10^7$
Prep. IX, murine neurovirus .....	0.32
» XXVIII, intestinal material from cases of poliomyelitis .....	0.12
» XXXVI, intestinal virus from mice .....	0.11
» XXXIV, intestinal material from brown rats .....	0.11

by Bawden and Pirie (8) and by Stanley (143), and similar figures have been found for tomato bushy stunt (103), potato X (99), and tobacco necrosis virus (122). The plant viruses, therefore, seem to have the specific gravity of the proteins. — The animal viruses are not equally well examined in this respect. The large viruses seem to have a density of between 1.15 and 1.20, while those below 10  $\mu$  tend to approach values of about 1.30 (34). Beard and his collaborators (37) give the figure 1.19 for the specific gravity of equine encephalitis virus, which is of a certain interest, as this virus is neurotropic like that of poliomyelitis.

The ordinary methods for determination of specific gravity, the pycnometric or the floatation methods, were hardly applicable in the present case on account of the scarcity of material. It was therefore decided to calculate an approximate value from the rate of sedimentation in glycerin solutions. By the formula for correction to the density and viscosity of water the specific volume was computed from the sedimentation constants of table XXIII. Assuming the viscosity of 30 p. c. glycerin  $\eta = 2.5$  and the specific gravity  $\rho = 1.073$  the value  $V = 0.76$  is obtained, corresponding to a specific gravity of 1.32.

This value refers to the  $\alpha$  component of human stools. Taken by itself it is obviously of little significance. The rate of sedimentation has proved to be dependent upon the concentration, and it can not be regarded as self-evident, that this relation should be independent of the medium. Furthermore it is not known whether or not dissociation or aggregation of the substance might occur in glycerin solutions. With these reservations it tends to show, however, that the  $\alpha$  component is protein in nature, i. e. in the same direction as the rest of the evidence. It must therefore be regarded as very plausible

Thus in the following calculations the specific volume was assumed to  $V = 0.76$  for neurovirus and the intestinal components alike.

The sedimentation constant of the pure and presumably dehydrated neurovirus protein was found to be  $181 \times 10^{-13}$ . On the basis of this value and the diffusion constant  $0.32 \times 10^{-7}$  the molecular weight was calculated to  $57 \times 10^6$ . Computing the axial ratio of the molecule in the ordinary way one arrives at the value 38:1. The formula for this computation is developed for the case of ellipsoidal and non-hydrated molecules. It is hardly probable that molecules of the shape indicated by the above axial ratio should be ellipsoidal. It is more plausible that the particles are filamentous or composed of shorter elements associated end to end, in which case the shape would be closer to that of a cylinder. The theory for sedimentation of cylindrical particles has not been developed. When the axial ratio is large as in this case, one might assume, however, that ellipsoidal and cylindrical particles of the same mass and the same length would have approximately the same coefficient of molar friction. On this assumption the dimensions of the particles were calculated to about  $12.5 \times 580$  m $\mu$ .

Under the same conditions, using the sedimentation constant  $220 \times 10^{-13}$  and the diffusion constant  $0.11 \times 10^{-7}$ , the molecular weight of the intestinal component was found to be  $200 \times 10^6$ , the axial ratio about 145:1, and the size of the particles  $12.2 \times 2150$  m $\mu$ .

### Stream double refraction.

As an attempt to check up on the above figures a specimen of human intestinal material was examined by Snellman for stream double refraction with the technique described by him (139).<sup>1</sup> The solution showed no spontaneous birefringence but already at very low velocity gradients stream double refraction appeared. As the apparatus was not constructed for such low velocities exact measurements could not be made. The result was, however, indicative of particle lengths in the range of 5–6000 Ångström units. Furthermore it was evident that the material was rather inhomogeneous with regard to particle size.

It should be remarked, that spontaneous appearance of a bire-

<sup>1</sup> These experiments were carried out at the Institute of Physical Chemistry, Uppsala.

fringent bottom layer, as happens in sufficiently pure and concentrated solutions of tobacco mosaic virus, was not yet observed in solutions of neurovirus or intestinal material; it may be that neither the degree of purity nor the concentration were suitable for the occurrence of this phenomenon.

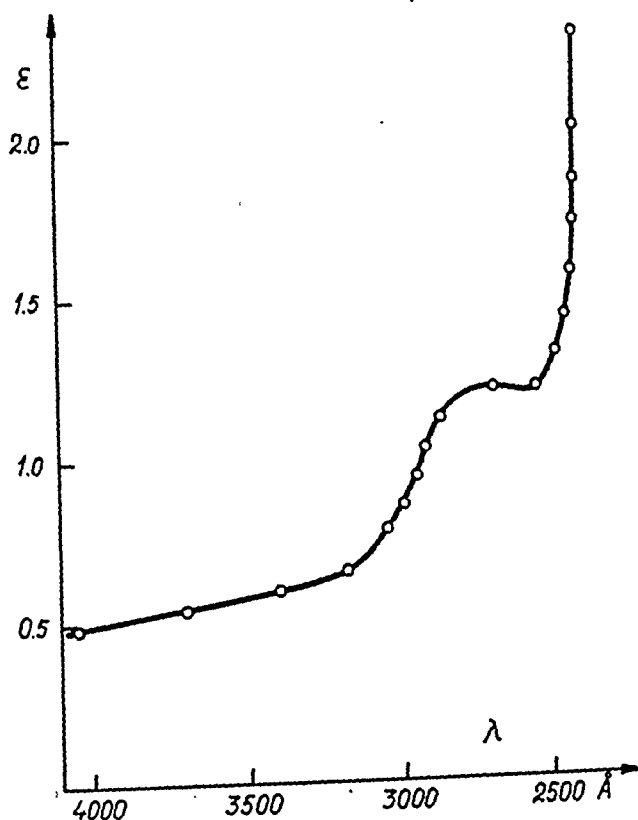


Figure 25. Ultraviolet absorption of purified human intestinal protein.

### Ultraviolet absorption.

In these experiments a Judd Lewis spectrophotometer was used. As already mentioned the preparations of intestinal material showed a strong Tyndall phenomenon. In a number of experiments it was found, as could be expected, that the solutions showed a pronounced diffuse absorption in the ultraviolet, increasing towards shorter wave lengths. When it was attempted to eliminate this disturbance by dilution it was found that the sensitivity of the instrument was insufficient for recording the specific absorption. Finally, however, a preparation was obtained from normal human stools, which was

practically colourless and with a comparatively faint Tyndall cone (preparation XLVI). When examined in the spectrograph it gave the absorption diagram shown in fig. 25. This diagram is far from ideal. There is a very pronounced diffuse absorption, making the evaluation of the specific absorption difficult. Chances are that it should be interpreted as a normal protein spectrum rather than that of a nucleoprotein, as the maximum between 2600 and 2700 Ångström might be a false one, provoked by the Tyndall absorption. For definite conclusions examination with a more suitable equipment is necessary.

On the same preparation nitrogen and phosphorus determinations were performed. It was found to contain 16 p. c. nitrogen and 0.6 p. c. phosphorus. As a total of but 2 mg of substance was available, duplicate determinations were out of question. The error, therefore, might amount to  $\pm 10$  p. c. At any rate the figures suggest that the substance be a nucleoprotein with approximately the same nucleic acid content as the rodshaped plant viruses.

## II. Serological experiments.

Rabbits were immunized by intravenous injections of purified intestinal protein at 5 days' intervals. They each received a total of about 15 mg protein in saline solution, administered in 5 injections. Samples of blood were drawn through heart puncture before the immunization and one week after the last injection. The serums were centrifuged in an angle centrifuge at 3000 r. p. m. for 1/2 hour, frozen and stored without preservatives at  $-16^{\circ}\text{C}$  until used. Three rabbits were immunized, one with protein from cases of human poliomyelitis, another with a similar preparation from young mice, the third one with protein from brown rats.

Complement fixation tests were carried out in the usual way. A purified intestinal protein, the same that had been used for immunization served as antigen. When this was titrated in decreasing amounts against a constant quantity of complement and a constant concentration of normal serum on the one hand, hyperimmune serum on the other, a very substantial non-specific complement fixation was observed. The range between specific and non-specific fixation was too narrow to allow any clearcut results of a titration

of serum. The experiments were repeated with newly prepared proteins with the same results.

Precipitin reactions were carried out as follows. 0.5 ml amounts of a suitable dilution of serum, usually 1:10, were mixed with an equal volume of antigen solutions of decreasing concentrations, kept at + 50° C for 1/2 hour and further over night in the ice box. Readings were made with a hand lens magnifying 5 times.

With normal serum visible reactions were never obtained. With hyperimmune serum and the homologous antigen a thin, loose flocculation appeared after about 1/2 hour at 50° C. The precipitate was easily broken up at gentle shaking, after which reformation required another 1/2 hour. It hardly settled in the ice box. The reaction was visible in antigen concentrations down to about 1:200 000. The flocculation was decidedly of the type usually associated with filamentous antigens (H-type).

Each serum gave positive reaction with all of the three antigens. No significant differences in titer were observed, but the quantities of precipitate differed, indicating possible antigenic differences. Judged from this basis the proteins from human and mouse feces appeared identical while the protein from rats differed. Absorption experiments were started but gave no conclusive results.

The anti-human serum was tested against a number of preparations from normal stools, of different degrees of purity. When purification and concentration were carried far enough, the preparations gave consistently positive reactions. Hereby were, however, three different types of flocculation observed. Most often the one described above occurred. The precipitate formed seemed to be almost colourless even in dark brown solutions. Sometimes another kind of H-flocculation appeared. The precipitate was coarser, settled readily and was usually coloured, leaving a clear and colourless supernatant. On a few occasions finally, the O-type of precipitation was observed. In these cases the solution appeared milky and turbid immediately after mixing of the reagents. The precipitate was white, granular, and settled in compact masses.

On account of these observations it was felt that the serum contained heterologous antibodies as well, probably originating from impurities present in the preparation used for immunization. Attempts were made to remove these antibodies through absorption. It was soon found, however, that the fractions used for ab-

sorption still contained considerable quantities of the homologous antigen. The result of the absorption experiment tended, therefore, to complete extinction of the precipitin reaction. As it would probably be easier to obtain a pure homologous antigen for immunization than pure heterologous fractions for absorption, the attempts to obtain a strictly monospecific serum were abandoned for the time being.

Until now only one experiment with neurovirus has been performed. A dilution of preparation XXXI, human neurovirus, was mixed with antihuman serum. No flocculation was observed. It was, however, questionable whether the concentration of virus protein in this case exceeded the critical level for visible reactions. The question of the serological relationships between neurovirus and the intestinal protein still remains open.

Neutralization experiments with the hyperimmune serum have not yet been carried out.

So far the serological experiments have not yielded any practical results. The description of this work was included, however, to show that the substances studied possess antigenic properties. It is therefore very probable that a continuation and extension of these experiments will provide more substantial evidence concerning the nature of the intestinal proteins, about which at present only vague theories can be presented.

### III. Electron optical studies.

A number of purified specimens were studied in the electron microscope at Laboratorium für Übermikroskopie, Siemens und Halske A.-G. in Berlin. The results have been reported elsewhere (152, 48, 49) and will be only briefly summarized in the following pages. A first series of specimens including the numbers 1), 3a), 4), and 5) (see below) were examined by professor A. Tiselius, the remaining specimens by the present writer.

The commercial type of the Siemens und Halske electron microscope was used, and the technique employed differed in no way from the ordinary (127). All preparations were dissolved in distilled water. Series of tenfold dilutions in distilled water were prepared. Of each dilution a small drop was placed on the object carrier and



The fluid balance between the blood in the cellophane casing and the solution outside it may be obtained in two different ways: a) by adding quantities of a suitable colloid to the salt solution until its colloidal osmotic pressure is equal to that of the blood, b) by compensating the colloidal osmotic pressure of the blood by corresponding hydrostatic pressure.

### 1. Colloids.

When a colloid is added to the salt solution for dialyzing blood *in vivo*, the colloid is not administered intravenously, it is true, but the contact is indirect via the cellophane membrane. However, large quantities of blood come into such indirect contact with large quantities of salt solution (when treating homo, about 100—200 litres of blood to 200—250 litres of salt solution per uninterrupted treatment). Such colloids as are available in the open market and can be regarded as applicable for this purpose are, however, not uniform but contain molecules of different sizes. They ought therefore not to be used for the dialytic treatment of human beings before they have been most carefully tested on animals and, if necessary, cleansed of low-molecular compounds that might pass through the membrane, exercise a toxic effect on, or remain deposited in the organism of the individual undergoing treatment.

Besides the above disadvantages there are others that will be dealt with in the following.

In this respect our studies are confined to the dialysis of blood against colloidal salt solutions *in vitro* and to a limited extent to experiments on rabbits. We have tried *gummi arabicum* (acacia) and *amylum solubile*.<sup>1</sup> As was only to be expected, we had no difficulties in maintaining the fluid balance between the blood and the salt solution + colloid. The following experiences are however worthy of mention: a) These substances contain cristalloids of varying or non-physiological concentration; our preparation of *gummi arabicum* contained large quantities of calcium and *amylum solubile*, large quantities of phosphorus, b) The strong formation of froth made it difficult to control the pH value of the salt solution by means of sodium bicarbonate +  $\text{CO}_2$ .

<sup>1</sup> By the courtesy of the Research Laboratory L. K. B. (polyvinyl-alcohol) and A/B Ferrosan (*amylum solubile*) who placed preparations at our disposal free of charge, it was possible for us to carry out this part of our research work.

c) Analysis of the substances dialyzed from the blood into the salt solution is rendered difficult. d) It is difficult to obtain sufficient quantities of colloids; colloids with a satisfactory degree of purity would be far too expensive in practice.

The above also applies essentially to polyvinyl-alcohol.

*The present apparatus affords the possibility to maintain the fluid balance by means of hydrostatic pressure. As it is possible even to obtain a stronger dehydration in the modified form of the apparatus already mentioned by one of us (A. 1947), we have no »colloid» problem. We have therefore discontinued to work on it.*

## 2. Hydrostatic Pressure.

The cellophane casing of the apparatus contains a limited and definite amount of blood, which is practically independent of the pressure in the casing. By suitably adjusting the pressure it is possible to maintain equilibrium between the blood flowing through the casing, and the surrounding salt solution. We have studied the fluid balance both in vivo and in vitro.

*In vitro:* The fluid balance between heparinized animal plasma and a salt solution has been studied in the following manner.

A relatively small dialysis apparatus was used for the experiments. The plasma was made to flow from a container placed about 1.5 m above the level of the apparatus. The rate of flow was controlled by a valve described in an earlier publication (A., 1947). All conditions were constant except for the fact that the height of the rubber tube, through which the fluid left the apparatus (= the hydrostatic pressure) was changed. From this outlet tube the plasma was allowed to flow into a vessel, whence it was poured back into the first mentioned high container. In this manner about 200—250 ml. plasma circulated through the apparatus at a rate of  $3/4$ —1 litre per hour during six hours. The experiments were carried out at room temperature.

Table 1 shows *firstly* the length of the outlet tube (= the hydrostatic pressure exercised against the contents of the casing during the major part of the experiment) and *secondly* the change of the protein content of the plasma during 6 hours' dialysis as determined according to Van Slykes copper sulphate method.

As was to be expected, we found that at the low hydrostatic pressures, the protein content of the plasma fell — *i. e.*, fluid flowed from the salt solution to the plasma — whilst the protein content remained constant or increased a little with the higher pressures.

occurs. The arrows in the picture indicate details of the structure, which must be interpreted as the ends of individual fibres of a bundle. On account of this condition statistical evaluation of the fibre width was not attempted. Instead of that measurements were carried out on a number of micrographs showing presumably single fibres not stretched. Thus a mean value of about 15  $\mu$  was obtained, from neurovirus and intestinal proteins, from human and murine specimens alike. As the width of the fibres is near the resolving power of the instrument, the accuracy of the measurement is probably of the order of 2—3  $\mu$ .

Observations on specimen 3b) in different states of purity revealed the following facts. The impure preparation contained beside the filaments two morphologically distinct components. One consisted of rounded particles 50—100  $\mu$  in diameter. The other one was finely dispersed, the elementary particles just about visible, i. e. below 10  $\mu$ . The filamentous phase consisted almost exclusively of single fibres in netlike arrangement. The particulate impurities were enmeshed in this network, while part of the amorphous material seemed to be adsorbed onto it. With increasing degree of purity first the particulate phase disappeared, then gradually the amorphous phase as well. In the purest preparations only the filamentous phase was visible. From comparison with the sedimentation diagrams it was concluded that the large particles corresponded to the  $\gamma$ , the amorphous substance to the  $\beta$  component. Parallel to the degree of purity the arrangement of the fibres changed. An increasing tendency to formation of bundles was obvious. The purest preparations contained hardly any single fibres except as short branches of thicker bundles. One got the impression that the impurities enmeshed in the network prevented association of the fibres mechanically; when these were removed a sort of coagulation of the gel structure ensued.

When the micrographs of the different specimens were compared on the basis of these general observations the following characteristics were found.

The intestinal proteins showed no differences that could not be referred to the actual state of purity. The fibres and their arrangement in active and inactive preparations were identical, and no further elements that could explain the biological activity were observed. Likewise no significant dissimilarities between human, mouse and rat proteins were detected. The sizes of the single fibres

varied considerably; they seemed, however, to be more uniform in the impure specimens. The meshes of the netlike gel structure were of an order of magnitude of about  $1\ \mu$ .

The micrographs of murine neurovirus showed mainly long filaments, almost exclusively single fibres in a loose network with wide meshes. Now and again impurities appeared to be adsorbed to the fibres, forming bead-string-like structures. The tendency to bundle formation seemed to be less pronounced.

In the human neurovirus preparations a finely dispersed substance predominated. It seemed to be rather uniform with regard to particle size and shape, forming rounded elements of about  $10\ m\mu$  in diameter. In the bulk of this substance single fibres were embedded, sometimes branched but seldom in netlike arrangement. Bundles were never observed. A third component in the shape of rounded particles might have been present, very difficult to distinguish from aggregates of the main component.

It should be pointed out that many of the details now described might be artificial, appearing during the process of desiccation and, therefore, not significant for the natural state of the solutions used for preparation of the objects. For instance, free filaments must always appear in a netlike arrangement, if no forces of orientation come into function during the desiccation. In the present case overlapping of fibres was very seldom observed, the ends being consistently apposed to the neighbouring fibres. This indicates that the net formation was not entirely artificial. Probably a netlike gel was formed when the solution became sufficiently concentrated through evaporation. In the diluted specimen on the other hand, the fibres must have been free.

There is certain evidence to show that the formation of bundles might be entirely artificial. On a number of micrographs from highly purified protein solutions, elsewhere showing pronounced bundle formation, a striking parallel orientation of single fibres was observed. As no signs of tension of the carrier film indicative of an orientation after fixation to the film were visible, it must be assumed that the orientation had taken place during the desiccation process. If the end of a fibre sticks to the carrier film, and the fibre is then caught by the edge of the fluid during evaporation receding from the hydrophobic film, it will be orientated perpendicular to the edge of the fluid. This was probably what had happened in the objects just

mentioned. If this be the case, it indicates that the fibres were not associated to bundles in the solution. The reason why bundle formation appears only in the pure preparations is probably simply that in this case no impurities prevent the complete collapse of the gel structure.

Finally the interpretation of some micrographs as indicative of adsorption of amorphous material on the fibres is of course open to the same criticism. No observations bearing upon the real implication of these pictures were made.

\* \* \*

The sedimentation and diffusion constants of the different preparations indicate a pronounced dissymmetry of the molecules. The numerical values of the dimensions, calculated on the basis of the experimental data, must be considered uncertain and for several reasons. (a) The determination of the constants themselves was not sufficiently accurate. As the dependence upon the concentration, salt content of the solvent, etc. was very marked, the final values had to be estimated through extrapolation, a procedure inevitably involving considerable sources of error. (b) The material was inhomogeneous, and all figures were consequently to be regarded as average values. (c) Finally the method of calculation was valid under certain conditions only. — On that account too much weight must not be attached to the exact figures. Their order of magnitude is, however, significant. Accordingly the particles are to be regarded as slender rods or fibres, probably of a uniform width of 12–13  $\mu$  their length on the other hand varying considerably. This indicates that the particles are aggregates of smaller units in chainlike association. From the sedimentation data it can be inferred that the tendency to chain formation increases with the degree of purity.

This conception was corroborated by the stream double refraction measurements and above all by the electron optical observations. The estimation of the length of the elementary particles, arrived at micrographometrically, needs further confirmation, however. By application of appropriate methods for preparation of the microscopical objects it might also be possible to study the dissociation phenomenon discussed in chapter IV.

The chemical analysis of the  $\alpha$  component is decidedly in favour of the conception of this substance as a nucleoprotein. In the sequel it will be referred to as the high molecular intestinal protein.

## Discussion.

The aim of the present study was the isolation in pure state of the viruses of murine and human poliomyelitis. As a working hypothesis it was assumed that these two viruses were closely related, and that in experimental work one of them could serve as model of the other. Consequently all methods were worked out on the mouse virus and then directly applied to the human type.

By means of procedures generally adopted for purification of viruses — with slight modifications necessitated by the nature of the starting material — it was possible to isolate from infected mouse brains a substance, presumably identical with the virus protein. The basic arguments for this presumption were presented in chapter V page 109 and were as follows. (a) The substance was present in all active specimens sufficiently concentrated but absent in concentrates of extracts originating from normal mouse brains. (b) The preparations, representing but  $1.5 \times 10^{-4}$  p. c. of the starting material, had retained about 30 p. c. of the total virus content. Thus the viral activity of the substance was about 200 000 times that of infected brains. (c) The activity of different preparations was proportional to the content of this substance. (d) By sedimentation analysis the purest preparations proved to be homogeneous with a sedimentation constant equal to that of the virus as determined by biological recording.

As previously mentioned the size of the virus as determined by ultrafiltration was estimated to 10—15 m $\mu$ , which in case of asymmetric shape of the particles refers to the small axis. In agreement with this figure the molecules of the virus protein were found to be rodshaped or filamentous, the width being 12—13 m $\mu$  as determined indirectly from sedimentation and diffusion data or about 15 m $\mu$  in electron optical measurements.

In chapter IV the statistical evaluation of titration data led to the conclusion that most of the virus particles appeared in larger aggregates, and that these aggregates probably dissociated on dilution. Sedimentation and diffusion experiments disclosed a certain inhomogeneity of the presumed virus protein, indicating that the particles in reality were aggregates of smaller units associated end to end. This was confirmed in the electron microscope, where the length of the elementary particles was estimated to about 115  $\mu$ . Single virus molecules were scarce, most fibres having a length of about 1  $\mu$ , corresponding to 8 or 9 molecules. There was, however, evidence to show that the method for preparation of the dried films favoured aggregation of the particles. It is, therefore, probable that the average of 580  $\mu$ , calculated from the sedimentation experiments, and corresponding to 5 elementary particles, is more correct as an estimate of the length of the chains when in solution. For the same reason it could not be decided whether the aggregation was reversible by dilution.

As developed in chapter IV it must be assumed on anatomical grounds that one MID as a rule comprises more than one active virus particle, aggregated or not. The actual number is dependent upon the relative amount and the distribution of susceptible tissue, which was indicated by the introduction of the resistance factor  $p$ . The anatomical structure of the brain and the probable refractoriness of the glial elements considered, one must admit  $p = 0.01$ , corresponding to 100 particles per MID, to be a plausible value. The molecular weight of the virus protein was calculated to about 57 000 000, referring to aggregates of probably 5 elementary particles, as pointed out above. On the basis of this figure and the last column of table XXII the number of aggregates per MID can be calculated to between 99 and 354.

All these observations give further support to the conception of the substance isolated from infected brains and the filamentous particles observed in the electron microscope as the virus itself.

On account of the minute yields of virus its chemical nature has not yet been studied. In experiment 20 a partially purified preparation was found to contain 0.069 mg nitrogen per ml. This corresponds to a nitrogen content of only 3 p. c. of the dry weight. From the sedimentation diagrams the virus content of this preparation was estimated to about 6 p. c. of the total solids, which calculated

as protein represents 0.023 mg nitrogen per ml. The impurities had obviously a very low nitrogen content, probably consisting largely of lipoids, and the result of the chemical analysis of this specimen, therefore, is not incompatible with the assumption of the virus as a protein.

A comparison with the plant viruses reveals several interesting similarities. Most of these viruses — the only exception so far being that of tomato bushy stunt — are rodshaped with a width of 10–15  $\mu$  and varying lengths. Tobacco mosaic virus is the one best studied. Preparations from young plants purified through differential centrifugation in distilled water are homogeneous with regard to sedimentation with  $s_{20} = 174 \times 10^{-13}$  (163). After exposure to salt solutions they become inhomogeneous with a second component of  $s_{20} = 200 \times 10^{-13}$ . Through viscosity measurements the axial ratio was estimated to 35:1 on the basis of which the dimensions were calculated to  $12.3 \times 430 \mu$  (96). The second component in the sedimentation diagrams corresponds to two such unit particles associated end to end. The diffusion constant was determined to  $D_{25} = 0.3 \times 10^{-7}$  (39).

In a recent paper Bernal and Fankuchen (14) reported an extensive study of the X-ray pattern of the virus. In orientated dry gels they found intermolecular spacings of about 150 Å, whereas in wet gels this distance had a minimum value of about 170 Å and varied with pH and salt concentration. They assume that the difference is accounted for by hydration of the molecules.

Kausche, Pfankuch and Ruska (81) measured the particle size on electron micrographs and found a constant width of about 15  $\mu$ , while the length showed a considerable variation. They found no rods shorter than 300  $\mu$ , and most of them held a multiple of this length. A few, however, measured an odd multiple of 150  $\mu$ , indicating that this should be the unit size.

In Wyckoff's centrifugal studies on plant viruses no observations concerning the influence of concentration upon the rate of sedimentation are mentioned. He uses throughout solutions of about 0.15 p. c. virus protein. Likewise no systematical investigation of the dependence upon the ionic strength of the solvent has been carried out. However, from the available data a certain trend of the figures is recognizable (163, 164). The rate of sedimentation seems to be lower in distilled water than in buffer solutions. Furthermore a



certain pH effect is visible, a maximum of sedimentation velocity coinciding with the isoelectric point, with a gradual decrease towards the limits of the stability range. This observation if verified is conform with the result of the X-ray study by Bernal and Fankuchen, and tends to show that the virus is hydrated. This means that the specific volume is larger than the value 0.77, derived from measurements on dried material. The size of the particles, therefore, is probably larger than that estimated by Lauffer. Assuming a degree of hydration as indicated by the X-ray crystallographic measurements one arrives at about  $20 \times 720 \text{ m}\mu$ . It is, therefore, very probable, that even the homogeneously sedimenting component of  $s_{20} = 174 \times 10^{-13}$  represents aggregates of molecules. The homogeneity might be only apparent, a result of the relatively high concentration of virus. It might be mentioned that the largely inhomogeneous intestinal protein in concentrations of 0.25 p. c. or more in distilled water sediments with abnormally sharp boundaries, the concentration gradients being too steep to be recorded by the scale method.

The electron optical observations on the particle lengths are of considerable interest. Although the existence of unit particles of  $150 \text{ m}\mu$  in length was probable, no such single units were detected. Most aggregates contained an even number of elementary particles, while those with an odd number were scarce. In this connection the dissociation formula C (chapter IV page 76) must be kept in mind. According to that formula even-numbered chains of molecules should predominate if the difference between the two dissociation constants were sufficiently large. From Bald's deductions (4, 5) it is evident that the deviation from the curve of normal distribution of takes is of exactly the same type in tobacco mosaic as in mouse poliomyelitis. In the case of tobacco mosaic, however, there is no way to determine the single molecule lesions and furthermore the probability factor  $p$  is of quite different order of magnitude — probably as low as  $10^{-6}$  —; why the dilution endpoint falls in another range of virus concentration. On this account it is not feasible to decide by biological methods whether one or the other dissociation formula is the more probable.

At any rate the neurovirus from mice seems to have many essential qualities in common with tobacco mosaic virus. Most characteristic is the tendency to aggregation of the particles end to end. On the other hand the electron micrographs disclose certain dissimilarities.

Single particles and shorter filaments of the tobacco mosaic virus appear very straight and rigid, whereas the neurovirus seems to be very flexible and elastic, in this respect more like potato X virus (81). This might explain why the tobacco mosaic virus readily forms parallel orientated paracrystals, whereas potato X — and probably the neurovirus — do not.

\* \* \*

When the methods of purification elaborated in experiments on the mouse virus were applied to nervous tissue from cases of human poliomyelitis, small amounts of a substance were recovered, showing a striking resemblance to the mouse virus. This substance was not obtained in pure state — in fact a complete purification was not attempted or even wanted. The yield amounted to a total of about 0.1 mg and as the purification procedure involved separation of the material in substance, it was felt that such small quantities might easily disappear during the manipulations unless some sort of a carrier substance was used. The high molecular weight component present also in extracts of normal nervous tissue served as carrier. It can not be excluded that the presence of this constituent interfered with the rate of sedimentation of the faster component. In preparations of the mouse virus, however, the presence of similar slowly sedimenting material effected no alteration of the sedimentation velocity of the virus. It seems, therefore, justifiable to operate with the values recorded.

The sedimentation constant of this substance was determined to  $s_{20} = 150 \times 10^{-13}$ , i. e. exactly the same as that of murine virus of the same degree of purity. In the electron micrographs structures of the same shape and size as the mouse virus appeared, embedded in a mass of amorphous material, the carrier substance. Apart from these similarities, the facts that the one preparation tested in a monkey was active, and that the substance now in question was not recovered from normal brains, suggest that it represents the virus protein.

The present study, therefore, has presented further suggestive evidence of the close relationship between the human and the murine poliomyelitis. The working hypothesis, according to which the murine virus and the mouse disease served as model of the human type, has been corroborated.

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The small yield of virus protein obtained from infected nervous tissue necessitated the search for other sources of virus. For this purpose feces from mice and cases of human poliomyelitis, known to contain comparatively large amounts of virus, were most close at hand. As starting material they had certain obvious disadvantages, but were never the less preferable from many points of view. This kind of material would probably be easier to purify; it is available in large quantities — and cheap.

This part of the investigation has hardly passed the preliminary stage. The experimental evidence is still incomplete and in drawing conclusions one must proceed with the greatest caution. In the writer's opinion, however, it offers an interesting point of departure for future work, and it was therefore included in this report.

Before the nature of the high molecular intestinal protein is discussed a few remarks on the purification procedure and the choice of methods should be made. As already mentioned it was observed that tobacco mosaic virus, originally homogeneous, after exposure to salt solutions became inhomogeneous. Parallel with increasing inhomogeneity the rate of sedimentation increased, the stream double refraction indicated increased particle length, the filterability decreased, and the activity decreased. On this account the ammonium sulphate precipitation was regarded as too drastic and inferior to the mild differential centrifugation method.

Bawden and Pirie (8) found that the ability to form liquid crystals depended upon the degree of purity of the virus solution. Bernal and Fankuchen (14) stated that the decisive factor must be the length of the particles, and advanced the theory that low molecular impurities were attached to the ends of the rods, preventing them from associating to longer fibres. As an indirect confirmation of this hypothesis Kausche (80) was able to produce in purified virus solutions real crystals of the same type as those observed *in vivo* by addition of crude plant juice.

As a corollary of this theory the tendency to aggregation would be a measure of the purity of the solution, and inhomogeneous sedimentation rather to be welcomed than avoided as evidence of successful purification. The effect of salt solutions might be that of elution of impurities adsorbed onto the virus molecules and inseparable from them by mere differential centrifugation. As pointed out on page 124, the experience with the intestinal protein strongly

favours this assumption. The serious problem is whether the treatment with salt has any deleterious effect on the activity. The lesion count is simply a method by which the relative number of infective aggregates is estimated, no matter whether each aggregate contains one or more active virus units. It is therefore quite natural that the activity per unit weight thus measured should decrease with increasing aggregation. This, however, does not necessarily mean that the virus is partially inactivated. The crucial test, reestablishment of the original activity after redispersion of a purified solution, has not yet been carried out.

The incubation time titration method in mouse poliomyelitis is probably independent of the degree of dispersity of the virus. The mouse virus would, therefore, be a suitable test object in experiments concerning the nature of the salt effect. No direct tests to that end were performed, but certain conclusions might be drawn from observations made in purification experiments. The yield of purified virus was about 30 p. c. as estimated from activity tests. In a few cases the different fractions, usually discarded, were systematically sampled and tested for activity. It was found that the loss of activity was approximately accounted for by the total activity of the discarded fractions. The deficit of 70 p. c. was therefore referable to a loss of substance rather than an inactivation of the virus.

Although incomplete as evidence this indicates that the ammonium sulphate precipitation does not damage the virus. The choice of purification method, therefore, should be dependent upon the ultimate aim of the procedure. Treatment with salt solution gives a purer product. If on the other hand preservation of the original physical state rather than chemical purity is wanted, differential centrifugation should be preferred. Concerning these filamentous giant molecules the paradox seems to be applicable, that the more inhomogeneous the purer is the material. It is also obvious that the sedimentation constant is of little value as a characteristic of such substances.

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As described in chapter VI a substance referred to as intestinal protein was regularly found in the feces of man and white mice, regardless whether active virus was excreted or not. It was also recovered from brown rats and pigs but not from white rats and gui-

nea pigs. With the methods used, sedimentation and electron optical analysis, it could not be distinguished from the virus protein. It was composed of molecules or particles of the same size and shape as those of the virus, possibly showing an even more pronounced tendency to aggregation; this, however, could be a secondary phenomenon and the result of a more extensive purification. In active specimens the viral activity was concentrated along with this protein.

From these observations it is evident that it was impossible to decide whether the substance isolated from infective specimens consisted of virus plus inactive protein, or should be regarded as qualitatively homogeneous.

Whichever be the case, it is hardly probable that the similarity between the virus and the "normal" intestinal protein is merely incidental. To the writer's knowledge substances of this type have not previously been isolated from material of animal origin, and those from plants possess viral activity without exception. This field is only just opened, that is true, and but little work has been done as yet.

Claude (27) and later also Furth and Kabat (41, 42, 43, 79) examined heavy fractions obtained by high speed centrifugation of organ extracts. Their preparations were largely inhomogeneous and seemed to consist of cellular debris of particulate nature, probably mitochondria. These particles were, however, of a different order of magnitude when compared with the protein molecules now in question.

Beard and his collaborators (12, 37) when isolating the viruses of rabbit papilloma and equine encephalitis, subjected normal tissue to exactly the same treatment. No substance whatsoever was recovered.

In the process of purification of the neurovirus as well as the intestinal protein a number of heavy components were observed. As revealed by the electron micrographs some of these were particulate, forming spherical bodies of different sizes. Others were finely dispersed, the particle size below the resolving power of the microscope. Similar substances were obtained in control experiments from internal organs such as liver and kidney. However no components of the virus type were detected.

Several virus preparations of animal origin have been examined electron optically but so far no structures similar to those of the intestinal protein have been observed (v. Borries, pers. comm.).

Thus it is evident that substances of this type are at least not common. This circumstance together with the facts that the intestinal protein was recovered from feces of the three species known to contract spontaneously poliomyelitic infections and at least two of them harbouring the virus in their intestines, and also from rats, constant companions of man and pigs, but not from the two further species of animals examined, suggests a closer connection between the virus and this substance than a mere morphological identity.

The assumption that the intestinal protein is an avirulent or non-neurotropic variant of poliomyelitis virus is very tempting. The chemical analysis, showing that it is in all probability a nucleoprotein, supports this hypothesis. The observation that it enhances the activity of a virulent virus, just as do low virulent strains, might have a signification in the same direction. Finally there is some epidemiological evidence in favour of the theory.

From a purely statistical point of departure Petersen (119, 120, 121) has deduced formulas for the development and course of epidemics. His deductions deal with three type cases. (I) The infectious agent is suddenly introduced into a population of a certain average susceptibility. Transmission from one individual to another does not occur. To this type belong for instance the waterborne epidemics of typhoid fever. (II) The infectious agent after being introduced into a population is transmitted from one individual to another through contact, droplet infection or otherwise. Infection produces immunity. (III) The infectious agent is ubiquitous. The individuals are constantly exposed to infection but normally resistant. Under certain conditions a lowering of the general level of resistance occurs, resulting in appearance of disease.

A computation of the epidemic curves out from these assumptions lead to the following results. Type I gives a curve with high initial case incidence, gradually diminishing. Types II and III both give normal distribution curves, provided in the latter case that the individual resistance shows a normal distribution. The study of a single epidemic leaves, therefore, the decision as to type pendent. There are, however, corollaries to the formulas, which make definite conclusions possible after comparative studies of a number of epidemics. In cases of type II the course will be different in intensive and mild epidemics in the same population or in epidemics of the

same average rate of morbidity in large and small populations. Epidemics of type III are independent of these conditions, the only factor affecting the course being the rate at which the resistance changes.

By comparison with the official statistics from different countries Petersen found measles, influenza and other typical contact infections to belong to type II. The agreement between observed and calculated data was excellent. The epidemics of poliomyelitis on the other hand were decidedly of type III. Considering the seasonal distribution and the differences in this respect between the warm and the temperate climates, he searched for a correlation with some well defined climatical factors. He found that the peak of the epidemics occurs at a time of the year when the insolation decreases most rapidly. From the seasonal distribution he calculated the rate of the presumed change of resistance and found a close correlation to the amount of insolation at the corresponding time of the year and the corresponding geographical latitude. His conclusions are, that the virus of poliomyelitis is ubiquitous, and that the lowering of the resistance against infection is the important factor in the epidemiology of the disease. This decrease in resistance coincides with the maximum rate of decrease in the insolation.

The pivotal point of Petersen's deduction is of course the presumed change of the virus-host interaction. For the mathematical treatment it is of no significance whether this change refers to the host, the virus, or both. On the assumption that the decisive factor is increase in virulence of the virus rather than decrease in host resistance, Petersen's theory should be of the utmost importance for the interpretation of the findings reported in this study.

With all reservations for the imperfection of the evidence presented, the following hypothesis will be advanced. The intestinal protein is a virus and a normal inhabitant of the intestines. As to the actual site of infection no conclusive evidence is available. Judging from the experiments on mice the virus is present principally in the contents rather than in the wall of the guts. This suggests, that some intestinal microorganism is the actual host. From the contents virus is probably being continually absorbed by way of the lacteals, deposited in the lymph glands, and gradually destroyed. From this "passive invasion" a slowly increasing serological immunity follows, a process that is probably intensified by intercurrent infections,

which would explain why the neutralizing substances develop earlier in urban than in rural populations.

Normally this virus is non-neurotropic, and as it does not possess avidity to any other tissues of the organism, its presence can not be revealed by our present biological tests. From a pathological point of view it must be characterized as not only apathogenic but non-infectious. Under certain conditions, however, the formation of neurotropic variants is favoured. In the domesticated mouse, not exposed to any considerable seasonal changes of the environment, these conditions are established, when the animals are being weaned, no matter what season. In man they are somehow, directly or indirectly, connected with certain climatic factors.

The nature of the active forces and the mechanism of the process can be but conjectured. It is well known that certain external factors like radiation, chemical treatment, etc. favour the appearance of mutants. If the intestinal virus were confined to the intestines of the respective hosts the active agent causing mutation most probably would have to be introduced with the food. If on the other hand an intestinal microorganism is the real carrier of the virus, this vector could be supposed capable also of living outside its host, and thus to be exposed more directly to the hypothetical agent. Whatever the mechanism water would be the most probable vehicle. There already exists some experimental and epidemiological evidence to that effect. A transmissible agent, causing poliomyelitis in monkeys, was recovered by Kling (86) from suspected well water. Furthermore the connection between atmospheric precipitation and poliomyelitis (45) suggests that surface polluted drinking water be a vehicle of the virus or the transformation agent.

When the suitable conditions cease to exist, the neurotropism subsides, either through disappearance of the mutant strains or through a gradual decrease in affinity to nervous tissue. In favour of the latter alternative is the common experience, that newly isolated strains of virus often cannot be established in passage at all, or they display a gradual decrease in virulence, extinguishing completely after 3 or 4 passages. In some strains, however, the neurotropism becomes fixed, making the individual host a carrier for life.

According to this theory the poliomyelitic infection would be in a sense endogenic, although certain exogenic factors are involved as well. There are other virus infections that could be explained on a



similar basis. In connection with herpes for instance the endogenic origin of virus has been most ardently discussed. In disposed persons the herpes eruptions can be produced at will by various stimulants, principally fever producing agents. Virus can be regularly recovered from the vesicles. In the meantime between attacks, on the other hand, it is usually not possible to detect the virus anywhere (for references see Doerr (30)). And yet it must be present in the tissues permanently. — Shope (138) produced conclusive evidence to show that the virus of swine influenza is transmitted by the lungworm, the eggs of which are excreted with the feces and taken up by earthworms. These in their turn are devoured by the swine, the lungworm develops in the intestines, penetrates the wall, wanders to the lungs, and the cricle is shut. That the virus is transmitted by this route is proved beyond doubt. Yet it has not been possible to show the existence of the virus in the lung worm in any stage of its developmental cycle. This must mean that the virus does not develop pneumotropic properties until the vector has reached the lungs of the host. As the pathogenicity of the virus is the only characteristic by which it can be biologically identified the non-pneumotropic variant cannot be detected.

Another interesting parallel can be drawn. The investigations by Claude (25, 26, 27) have revealed that the tumor producing agent from Rous' sarcoma is inseparably connected with certain particulate structures in the tumor extracts. The size of these particles is in the range of 50—200 m $\mu$ . They were found to consist of a nucleoprotein and about 50 p. c. phospholipoids. However, particles of the same size and the same chemical composition were recovered from normal tissues as well. To these particles are attached the heterogenetic antigen (41), the Wassermann antigen (42), and certain enzymes (79). They are regarded by Claude as preformed cellular structures, probably mitochondria. — In this case it is hardly appropriate to denote the particulate component of the tumor extracts as the virus of Rous' sarcoma. The tumor producing agent is combined with the mitochondria and its activity obviously intimately connected with the maintenance of the structure of these particles. Although pathologically altered they are nevertheless cellular constituents and might perhaps be regarded as mutant mitochondria.

The virus of poliomyelitis, on the other hand, is not a constituent of the cell. No similar substance or structure is present in normal

cells. However, the theory cannot be worked out in detail at present. The supporting evidence has obvious defects, and consequently the next objects of research are given: the chemical and serological relation between the neurovirus and the high molecular intestinal protein, the origin of the latter substance, and eventually the conditions necessary for its transformation into a neurotropic variant.

## Summary.

The aim of this study was the purification of the viruses of mouse encephalomyelitis (Theiler's disease) and human poliomyelitis.

Chapter I contains a review of the systematical position of the viruses in question. The virus of encephalomyelitis enzootica suum (the Teschen disease) is also included in the survey. The similarities between these three viruses are stressed. On the basis of the discussion a more uniform nomenclature is suggested, using the name poliomyelitis virus as species denomination, with human, murine, and porcine strains as subspecies or types.

The second chapter is an account of the general methods applied and the material used. Attempts at tissue culture of the mouse virus were without success.

In chapter III the methods for measurement of virus activity are reviewed. It was shown that the mouse virus can be propagated serially in the developing chick. The infection was restricted to the central nervous system, the final virus concentration remained on a low level, and no characteristic lesions were produced. The membrane technique, therefore, was not applicable to poliomyelitis virus.

The incubation time titration method was studied with special regard to the sources of error. The dependence of the incubation period upon the amount of virus inoculated, the volume of the inoculum, the weight, sex and age of the test animals was analysed. A linear relation was shown to exist between the reciprocal of the harmonic mean of the incubation time and the logarithm for the dilution. The slope of the titration curve was dependent upon the volume of the inoculum and the age of the test animals. Finally the accuracy of the method was estimated and its field of application discussed.

The accuracy of the 50 p. c. endpoint method and the relation of the titer-values to those obtained with the former method was analysed.

In chapter IV the theoretical basis for the interpretation of titration data is surveyed. Parker's experiments on the virus of vaccinia are discussed and a different interpretation suggested, based upon the assumption, that the virulence of a certain strain of virus is connected with the presence in the elementary bodies of an activator, necessary for the incitement of infection.

An identical experiment was carried out with the FA strain of mouse poliomyelitis virus. An analysis of this experiment showed that a group of animals could be recognized, in which the infection was probably incited by a single virus molecule. This observation formed a basis for the further discussion, which led to the conclusion that the virus must appear largely as aggregates of elementary particles, and that this aggregation might be reversible on dilution.

The same observation was very valuable as a point of departure in attempts to explain the connection between the amount of virus inoculated and the ensuing incubation period. It was assumed that an inhibitory influence upon the multiplication of the virus is exerted by intact ganglion cells and also transmitted to neighbouring cells. The degree of this cellular resistance against infection might be connected with the intensity of metabolism of the cell.

Chapter V gives a description of the purification of murine and human virus from brain and spinal cord. Through a combination of salting-out and differential centrifugation, preparations were obtained from infected mouse brains, the activities of which on a weight-by-weight basis were up to 200 000 times that of the starting material. In sedimentation analysis all preparations concentrated above a certain limit showed the presence of a component with a sedimentation constant of the order of  $150\text{--}160 \times 10^{-13}$ . No such constituent was recovered from normal brains. The purest preparations were homogeneous in the ultracentrifuge. The rate of sedimentation increased with the degree of purity and was higher in salt solutions than in distilled water. The sedimentation constants recorded were: less pure material in distilled water  $s_{20} = 150 \times 10^{-13}$ , pure material in distilled water  $s_{20} = 162 \times 10^{-13}$ , in 0.05 M sodium chloride  $s_{20} = 181 \times 10^{-13}$ .

The sedimentation of the virus was studied in the separation cell by means of activity measurements. The sedimentation constant of the biologically active component was determined to  $s_{20} = 152 \times 10^{-13}$ .

The titration data revealed the presence in crude brain extracts of inhibiting substances not combining with the virus in vitro. They were destroyed by digestion with pancreatic juice. As they were not present in extracts of normal brain they might have some connection with the tissue immunity.

When spinal cords from cases of human poliomyelitis were purified according to the same method, small amounts of a similar component were obtained, which was not present in normal brain and spinal cord identically treated. The sedimentation constant was determined to  $s_{20} = 150 \times 10^{-13}$ .

The yield of substance averaged 0.6  $\gamma$  per mouse brain and a total of 0.1 mg from 11 human spinal cords.

In chapter VI the purification of feces and intestinal contents is described. The method of purification was essentially the same as that applied to nervous tissue. From human stools, preparations consisting of three main components were obtained. One of these resembled closely the substance obtained from infected nervous tissue. Its rate of sedimentation was dependent upon its concentration and the salt content of the solution. The limit value of the sedimentation constant was estimated to  $s_{20} = 165 \times 10^{-13}$  in distilled water, and  $s_{20} = 220 \times 10^{-13}$  in 0.05 m sodium chloride solution. A certain amount of impurities, probably attached to the main component in the form of an adsorption compound could not be removed through differential centrifugation in distilled water or dilute sodium chloride solution, but was eliminated by treatment with 0.4 m ammonium sulphate. A pure preparation of this component had retained presumably all of the original virus activity.

However, an identical substance could be isolated not only from all cases of poliomyelitis examined, but from healthy contacts and from normal controls as well. The yield was practically constant, about 1 mg per 100 g of solids, regardless of the origin of the specimen.

In feces from normal mice a component of the same type was present. The yield corresponded to an excretion of 0.01 mg per mouse and day. The pure preparations from young animals produced typical poliomyelitis when inoculated intracerebrally, the activity being 100—1000 times that of the starting material. The substance derived from old animals was inactive even if inoculated in large quantities.

The purified intestinal virus showed no interference phenomenon when inoculated together with the virulent encephalitogenic FA strain. Addition of the intestinal virus increased the activity of a FA preparation as would an additional amount of the FA virus itself. Preparations from old animals, in themselves inactive, had a similar, although less pronounced, enhancing effect upon the FA strain.

Feces from a number of other animal species were examined for the presence of similar substances. No such components were obtained from guinea pigs and white rats. Small quantities of a presumably identical substance were detected in the intestinal contents from three pigs, and large amounts were constantly recovered from the feces of wild brown rats. Repeated tests for activity of these preparations in monkey, brown rats, and white mice were negative.

In chapter VII some experiments on purified material are reported. The diffusion constant of the substance derived from infected mouse brains was determined to  $D_{20} = 0.32 \times 10^{-7}$ . The intestinal components from man, the white mouse, and the brown rat were found to have the same diffusion constant  $D_{20} = 0.11 \times 10^{-7}$ . On the basis of these values and the corresponding sedimentation constants, and on the assumption of cylindrical and non-hydrated molecules the following characteristics were computed. Material from nervous tissue: molecular weight  $57 \times 10^6$ , particle size  $12.5 \times 580$  m $\mu$ . Intestinal material: molecular weight  $200 \times 10^6$ , particle size  $12.2 \times 2150$  m $\mu$ .

A pure preparation of intestinal material of human origin was examined for ultraviolet absorption. It showed a relative maximum of absorption in the range of 2600–2700 Å and a practically complete absorption of wavelengths below 2400 Å. A chemical analysis of the same preparation gave a nitrogen content of 16 p. c. and a phosphorus content of 0.6 p. c. Thus, the substance was probably a nucleoprotein.

In immunization experiments on rabbits, the intestinal proteins proved to be antigenically active and specimens from man, the white mouse, and the brown rat showed serological relationships.

Electron optical observations on the purified materials revealed the presence in all specimens examined of filamentous structures of uniform appearance. The width of the fibres was estimated to about 15 m $\mu$ . The length varied considerably and seemed to be

dependent to a certain extent upon the state of purity of the specimen. The purer the material, the longer the fibres. Simultaneously a tendency to aggregation side by side could be detected. Through a statistical evaluation of the particle lengths it was assumed that the fibres were composed of a varying number of smaller units, the length of the elementary particles being estimated to about 115 m $\mu$ .

In chapter VIII the results are summarized and discussed. On the basis of the discussion it is concluded that the substance obtained from infected mouse brain must be regarded as the pure virus protein. A comparison with the plant viruses reveals many interesting similarities.

Likewise the corresponding component of preparations from spinal cords of human cases of poliomyelitis probably represents the human virus protein. The investigation has further corroborated the assumption of the close relationship between the human and the murine virus.

The purification methods are discussed. The salting-out procedure gives a purer product. The differential centrifugation preserves the original physical state of the material, but gives a less pure product.

In a discussion of the nature of the high molecular intestinal protein it is pointed out that final conclusions cannot be drawn, as the evidence is still incomplete. A theory is advanced according to which this substance is regarded as a virus, ordinarily non-neurotropic but under certain conditions developing neurotropic properties.

## Appendix.

1) *Hospital record numbers of patients from which material was collected.*  
 The letters denote: A = Medical School Hospital, Uppsala; B = Hospital of  
 Epidemic Diseases, Bollnäs; G = Hospital of Epidemic Diseases, Gävle;  
 S = Hospital of Epidemic Diseases, Stockholm; U = Hospital of Epidemic  
 Diseases, Uppsala.

Brain and spinal cord	F e c e s		
	Poliomyelitis cases		Controls
A 1722/41	S 2674/41	U 259/42	A 179/42
U 201/41	S 2685/41	U 260/42	A 226/42
	S 2686/41	U 261/42	A 378/42
B 121/42	S 2692/41	U 264/42	A 452/42
B 126/42	S 2696/41	U 265/42	A 464/42
B 133/42	S 2714/41	U 272/42	A 482/42
B 147/42	A 24/42	U 273/42	A 526/42
B 152/42	A 340/42	U 274/42	A 555/42
B 153/42	A 413/42	U 277/42	A 572/42
G 190/42	A 512/42	U 283/42	A 613/42
G 196/42	G 174/42	U 284/42	A 629/42
S 1893/42	G 179/42	U 288/42	A 670/42
U 329/42	G 180/42	U 291/42	A 671/42
	G 182/42	U 292/42	A 707/42
	G 184/42	U 297/42	A 1277/42
	G 185/42	U 301/42	A 1724/42
	G 207/42	U 319/42	A 1725/42
	G 209/42	U 320/42	A 1753/42
	G 211/42	U 325/42	A 1786/42
	G 214/42	U 332/42	A 2001/42
	G 216/42	U 344/42	A 2458/42
	G 217/42	U 346/42	A 2474/42
	U 252/42	U 347/42	
	U 253/42	U 363/42	
	U 258/42		

Material was collected from one further lethal case of poliomyelitis, the father of patient B 147/42, delivered to the Province Hospital, Bollnäs, but never filed in the records. Sections of the spinal cord from this case were examined microscopically and showed typical poliomyelitic lesions.



2) *Formulas used.*

Chapter III, page 55. Quotient of two non-correlated means

$$M = \frac{M_1}{M_2} + \frac{M_1}{M_2^2} \cdot \varepsilon(M_2)^2.$$

Standard error of quotient

$$\varepsilon(M) = \pm \sqrt{\frac{\varepsilon(M_1)^2}{M_2^2} + \frac{M_1^2}{M_2^4} \cdot \varepsilon(M_2)^2}.$$

Chapter VII, page 134. Correction of sedimentation constant to density and viscosity of water

$$s_{\text{corr}} = s_{\text{obs}} \cdot \eta \cdot \frac{1 - V \cdot 0.9982}{1 - V \cdot \varrho}$$

$\eta$  being viscosity of solvent,  $\varrho$  specific gravity of solvent and  $V$  specific volume of the substance.

Concerning the formulas for calculation of molecular weight, frictional ratio, and axial ratio, the reader is referred to T. Svedberg and K. Pedersen: *The Ultracentrifuge* (146 a).

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Table V.

*Development of the electrocardiograms of treated pellagrins.*

Nicotinic Acid					Nicotinic Acid + Thiamin					Nicotinic Acid + Yeast					Yeast					Yeast + Liver Extract					No specific treatment					Total
Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	Normalised	Improved	Unchanged	Worse	Total	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
5	1	3	—	15	—	1	—	—	1	2	2	2	—	6	2	1	—	1	4	—	—	—	2	2	—	—	—	1	1	29
Inconclusive																													3	
Total																													32	

With reference to the presence of coronary diseases, the pellagrins are recorded in groups according to age and sex in table III.

Table IV is a summary of the age and sex distribution of pellagra-cardiograms; 10 series of tracings included in table II are omitted, seven normal ones and three others influenced by coronary insufficiency. The table shows, that in the group of younger pellagrins, abnormal tracings are not less frequent than in the group above 50 years. Hence the statistical analysis confirms the fact derived from the individual examination of every pellagrin, that coronary disease played no major rôle in shaping the abnormal cardiograms.

Table V summarizes the development of the pellagra-cardiogram in treated pellagrins.

Table V refers to the cases of columns 3, 4, 5 and 6a of Table II. However, three of these patients are omitted, owing to incomplete data. From the remaining 24 patients the cardiographic development in 29 courses of treatment is registered. Some pellagrins are registered more than once, if treated at various times by the same or by different procedures, for instance with niacin only at one time, with niacin and yeast in a subsequent period.

Table V shows:

During treatment with nicotinic acid the cardiograms became normal only in a certain number of pellagrins, even if the other pellagrous phenomena disappeared.

In the other cases the normalisation of the tracings remained incomplete in spite of prolonged treatment.

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# PLATES

I—III

## Plate I

### Mouse poliomyelitis

- a) Animal experimentally infected with strain UF I. Partial paralysis of the hind legs.
- b) Section of brain cortex. Perivascular cuffing. (Strain UF I.) Magnification 100x.
- c) Section from the region of the fourth ventricle. Perivascular cuffing, glial reaction and round cell infiltration, neuronophagia. Plexus chorioidens intact. (Strain UF I.) Magnification 100x.
- d) Section of spinal cord. Perivascular cuffing and round cell infiltration in anterior horn. (Strain UF I.) Magnification 100x.
- e) Section of spinal cord. Neuronophagia. (Strain UF I.) Magnification 900x.
- f) Section of spinal cord. Perivascular cuffing, scattered infiltration, neuronophagia. (Strain FA.) Magnification 100x.
- g) and h) Animal experimentally infected with strain FA. Fit of »jitter-bug disease», clonic convulsions.
- i) and k) Sections of brain cortex. Intense reaction with perivascular cuffing and scattered infiltration. (Strain FA.) Magnification 100x.



### Plate III

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- a) Neurovirus from mice. Impurities attached to the fibres. Electron optically 22000: 1.
- b) Neurovirus from man. The finely dispersed »carrier substance» blurring the outlines of the fibres. Electron optically 24000: 1.
- c) Intestinal protein from mice. Gel structure in concentrated material. Electron optically 25000: 1.
- d) Intestinal protein from mice. Bundle formation. The arrows indicate ends of individual fibres. Scattered single and double molecules visible. Electron optically 23000: 1.
- e) Intestinal protein from mice. Carrier film disrupted, filaments under tension largely deformed. Electron optically 23000: 1.
- f) Intestinal protein from man. Impure. Particulate matter enmeshed in the network. Electron optically 27000: 1.
- g) Intestinal protein from man. Medium purity. Low molecular material adsorbed on the fibres. Electron optically 25000: 1.
- h) Intestinal protein from man. Highly purified. Bundle formation. Electron optically 22000: 1.

deficiency and in clinical pellagra, associated or not with cardiac symptoms, has been definitely proved.

*Absence of parallelism between the electrocardiographic alterations and other pellagra manifestations.*

In the pathogenesis of the pellagra-tracing, the absence of parallelism between the general trend of the disease and the cardiographic alterations is a further unsettled problem.

The fact has already been noted by Weiss & Wilkins (29). They observed severe heart failure with or without abnormal tracings and in other cases cardiographic alterations without cardiac symptoms; they noted similar irregularities with reference to anatomical myocardial damage. In the induced thiamin deficiency of rats Weiss, Haynes & Zoll (30) found in different experiences with the same animal various patterns of cardiographic deformations or absence of abnormalities.

We encountered the same lack of conformity in pellagrins. Actually we have never seen serious pellagra of longer duration without abnormal tracings; this could be anticipated, since pellagra cardiograms were present in three quarters of the patients; however, there was no relation between the presence or intensity of the cardiographic changes and the duration, severity or predominant localisation of the pellagra manifestations. During treatment there was mostly a parallelism between the electrocardiographic development and the general trend of the disease; however, we have reported several notable exceptions.

We assume, that these irregularities are due to the interaction of other B-factor deficiencies; deficiency of a second factor can foster as well as prevent the production of myocardial damage produced by a primary deficiency; this unexpected fact is shown by experiments of Follis, Orent-Keilles & McCollum (6), of Follis (4) and of Thomas, Mylon & Winternitz (27).

The question, if protein deficiency — a frequent condition in pellagrins — modifies the influence of B-factors on the cardiogram has not been systematically investigated. In a case of extreme hypoproteinemia, however, observed by Killian & Ingelfinger (12) the tracing was normal.

Moreover the speed of the depletion has a bearing on the clinical phenomena of a deficiency disease. This was experimentally shown by Swank and Bessey (25) in the thiamin deficiency





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AN INVESTIGATION INTO  
A YEAST-LIKE FUNGUS  
ISOLATED FROM PATIENTS SUFFERING FROM,  
OR SUSPECTED OF, PULMONARY  
TUBERCULOSIS

*By*

EINAR HOLLSTRÖM

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CHIEF: PROFESSOR JOHN REENSTIERNA, M. D.

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AN INVESTIGATION INTO  
A YEAST-LIKE FUNGUS  
ISOLATED FROM PATIENTS SUFFERING FROM,  
OR SUSPECTED OF, PULMONARY  
TUBERCULOSIS

BY

EINAR HOLLSTRÖM

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## Preface.

This investigation has been carried out at the Institute of Hygiene and Bacteriology at the University of Upsala. I now wish to express my sincere gratitude to its Chief, Professor John Reenstierna, for his having aroused my interest in the sphere of research within which my work lies.

I also wish to thank Dr. Nils Gellerstedt for the great trouble he has taken to examine my entire material of sections; Professor Fredrik Berg for his kindness in examining the ocular changes that had occurred in the test animals; Dr. Erik Hedvall for his assistance in examining the X-ray films; and Professor Robin Fähræus and Professor Torsten Teorell for their kindness in placing at my disposal photographic equipment and micromanipulator.

I also beg to thank the assistants at the Institute, especially Dr. Vilhelm Hallberg, for their help and advice; Dr. Arne Franzell for assistance in taking the photographs; Miss Elli Öhrvall for her meticulously carried out drawings, and Mr. William Savage for his translation into English.

Last but not least I wish to convey my thanks to the Swedish Anti-Tuberculosis League for defraying the bulk of the expenses of my research work, and to the Regnell Fund of the University of Upsala for its contribution towards expenses.

Upsala, in April 1943.

*Einar Hollström.*





## Introduction.

The class *Eumyces* or true fungi has been subdivided into minor groups, according to their methods of sexual reproduction, but there is one large group, the *Fungi Imperfecti*, about which no method of sexual reproduction has been demonstrated. It is to this group that most of the pathogenic fungi important in medicine, belong. Many attempts have been made to classify these fungi, partly through their growth under certain conditions, partly through their capacity to disintegrate carbohydrates, by their serological qualities, and by their pathogenity in animal inoculation. But as yet no satisfactory solution of this problem has been found. The chief reason for this is the extreme pleomorphism in the fungi in question, especially in cultures. The indefinite classification has entailed confusion in taxonomy. As regards tests at classification and taxonomy of the various yeast-like fungi, reference is made to monographs and major treatises on the subject in question, e. g. Lafar (64), Vuillemin (134), Castellani and Chalmers (23), Sartory (107), Benedek (8), and Conant (26).

Yeast-like fungi have by many authors been demonstrated in the human body, particularly in morbid conditions, and they have by many been shown to be capable of being the cause of such. It is of especial interest to note that a yeast-like fungus has been pointed out as the mother-organism of Koch's bacillus. This opinion, which was advanced in 1912 by Reenstierna (98, 103), was based partly upon findings of such fungus in different tuberculous material, partly upon his observations that the fungus in question (single cell culture) was able to produce acid-fast rods. Further investigations in this matter have been carried out by Gullberg (41) in 1933—1935, but these were broken off by his death. My interest in this sphere of research was then aroused and was further incited when, in the year 1935, I could establish in a tubercle bacillus culture the appearance of fungus cells some of which contained short acid-fast rods.

As our knowledge of yeast-like fungi, especially their capacity to produce acid-fast rods, must as yet be looked upon as rather circumscribed and limited, an investigation into the subject seemed to me as likely of being of some value.

The initial material for my research consisted of five strains of fungus of yeast-type, isolated from patients either suffering from pulmonary tuberculosis or under observation with a suspicion of such.

In the investigation I have wished to study in the fungus strains:

- a) some of their morphological, tinctorial, and biochemical properties, as well as their ability to produce acid-fast rods in culture;
- b) their pathogenity in intravenous inoculation into rabbits and their ability to produce acid-fast rods in the animal organism; and
- c) to investigate the pathogenity of the acid-fast rods obtained.

## CHAPTER I.

### Historical Survey.

Yeast-like fungi of various species occur very commonly in nature. As regards their occurrence in a healthy human being, opinions differ. Thus Todd (126) by way of example states that in 1937 he found in the mouth or throat of 1,000 healthy persons a fungus of yeast-type in 14.7 per cent, and Knighton (55) that in 1939 he had been able to demonstrate a similar fungus in 36.9 per cent of the oral cavities examined. Pijper (91), on the other hand, declares in a work in 1923, first by reason of his own experience, and second as the result of investigations in 1922, by Küster (63) and Bloomfield (14) as well as Wall (128) in 1923, that yeast-like fungus is no common micro-organism in the mouth and throat of healthy human beings. Even in other parts of the human body such fungus has been mentioned as normally occurring, in the ventricle and also in the whole intestinal region, in the skin, in the lachrymal fluid, etc.

A remarkably large literature exists about the occurrence of yeast-like fungus in morbid conditions, when the fungus has been looked upon either as a cause of disease or as a secondary invader. As is well known, Langenbeck (66), discovered in 1839 in thrush such a fungus, which Berg (10) a few years later proved to be the causative agent of this affection.

As is to be found in a survey by Plaut and Grütz (95), also animal experiments have been carried out, by several investigators, with fungus strains isolated from thrush, probably not belonging to one and the same species. — Cf. Tanner's and Dack's statement (125). — Regarding experiments with intravenous inoculations into rabbits, the following may especially be mentioned: 1896 Ostrowsky (87) could establish a rise in temperature (up to 41.2° C) with ensuing hypothermy (down to 33° C) which still existed at the animal's death within 7 days; 1897 Steiner (117) observed, besides mycotic changes of the pectoral and abdominal organs, even such in

the central nervous system; Buss (16) in 1913 showed that the fungus contained endotoxins and was able to produce ectotoxins; Krauspe (59) in 1927 also proved the existence of endotoxins and found that these made the animals cachectic. Although not stated that the yeast-like fungus used in his experiments (intravenous inoculations into rabbits) originated from thrush, the interesting establishment by Stock (119), in 1908, of the appearance of ocular changes in the test animals should be mentioned in this connection. The ocular changes were: Conjunctivitis folliculosa, focal iritis, cyclitis and chorioiditis disseminata, focal affections in the retina, with delimited retinal loosening (clinically the affection was reminiscent of retinitis proliferans), as well as perineuritis nervi optici.

As to other morbid conditions in which the occurrence of yeast-like fungi have been established — vide some recently published surveys by Mitchell (81), Baker and Brian (5), Benedek (8), Sjövall (115) — the following should here be mentioned: ulcer ventriculi, where in 1922 Frank (37) and Cafasso (17) found such a fungus in more than 50 per cent; faeces in tropical sprue, stated by Kohlbrügge (56) in 1901, by Anderson (1) in 1917, and by Ashford (3) in 1929; vaginal affections, skin diseases, heart lesions, and the central nervous system; corneal affections, demonstrated in 1898 by Stöwer (124) and in 1900 by Lundsgaard (71); lower eyelid (tubercloid changes but without tubercle bacilli), described in 1928 by Ferguson (33).

With regard to *lung affections*, numerous findings of various species of yeast-type fungus have been reported. Thus for example Stowall and Bubolz (121) state in 1929 that from the sputum of 100 patients with pulmonary conditions they had isolated 40 strains of yeast-like fungus forms. Many investigators have considered the fungus as the causative agent of the disease: In 1898 Artault (2); 1905 Castellani (18); 1913 Forgues (36); 1914 Mautner (76); 1915 Boggs and Pincoffs (15); 1917 Pijper (90) and Simon (114); 1919 Magrou (74); 1920 Castellani, Douglas, and Thomson (24), Chalmers and Macdonald (25), Jacono (49), and Nathan (86); 1921 Mendelson (79); 1922 Sartory and Moinson (108), Servet (113); 1923 Farah (31), Joeques and Simpson (50), Pijper (91); 1926 Nasso (85), Kotkis, Washowiak, and Fleisher (58); 1927 Wallace and Tanner (129); 1928 Galbreath and Weiss (39), Stowall and Greely (123); 1929 Grossi and Balog (40), Kurotechkin and Chu (62); 1930 Panayotatou (88), Stokes, Kiser, and Smith (120);

1931 Warr (130); 1933 Stowall and Bubolz (122), and Bergman, Danielsson, Ottander, and Schnell (11); 1934 Bakst, Hazard, and Foley (6); 1935 J., R., and Z. Flinn (35); 1937 Black and Eddy (13), Davis and Warren (29), R. and J. Flinn (34), Spreng (116), and others whose papers I have not had an opportunity to study.

Castellani's works (18, 19) of the years 1905 and 1910 have been looked upon as the first contributions of any importance to our knowledge of bronchial mycosis caused by yeast-like fungus. He elucidated there that the disease which was common amongst the tea-workers in Ceylon, and passed under the term of "teatasters' cough" and "tea-factory cough", was caused by a fungus, generally of yeast-type. Since then a large literature on pulmonary affections, which have been considered as being caused by such a fungus, has been published, and especially in recent years it has increased greatly but without anything like uniformity in the taxonomy. In the literature in the English language the term broncho-moniliasis has generally been used, inasmuch as the cause of the affection had been considered either the Genus *Monilia*, according to Castellani's and Chalmers' Table<sup>1</sup> (23) or according to Benham's (9) expression "monilia in a medical sense", i. e. all yeast-like, filamentous fungi. The term *Blastomyces* has been used especially by American pathologists to express a budding fungus of yeast-type. A good illustration of the deplorable confusion is Vuillemin's statement: "Il existe bien un genre *Blastomyces*, mais, quelque étrange que cette assertion paraisse, les *Blastomyces* ne sont pas de *Blastomycètes*."

So-called primary broncho-moniliasis has by Castellani been divided into three degrees of gravity, viz. mild, intermediate, and grave, and has in the main been described in the following way: In the mild type the patient's general condition is unaffected and there is no fever. The trouble consists of a slight cough with mucopurulent expectoration, often scanty and only in exceptional cases tinged with blood. With physical investigation at the outside the ordinary signs of bronchitis are found. The intermediate type is characterized by a slight bronchitis and slight fever of long duration. The more or less troublesome cough is frequently of a paroxysmal character, occurring in the mornings and at night, sometimes also moderate dyspnoea. In the grave form the symptoms, the same as the entire

<sup>1</sup> The definition of the Genus *Monilia* is, according to Castellani's and Chalmers' own statement, vague.

clinical picture, and many times also the X-ray picture, are in full agreement with pulmonary tuberculosis. The patient has acute fever attacks, progressive emaciation, night sweats, pains in the chest, troublesome cough, and sometimes dyspnoea, as well as repeated haemoptyses. The sputum is mucopurulent, of varying quantity, but mostly considerable. Anaemia exists frequently to a fairly high degree, occasionally also eosinophilia and some time an increase in the white corpuscles. With physical examination the same findings can be obtained as in advanced pulmonary tuberculosis. The X-ray picture may show pleural congestions, enlarged hilus shades, large cloudy infiltrations, generally in the lower parts of the lungs, but some times also in the apices. Cavities are also occasionally met with. The different symptoms and findings by investigation may vary to a high degree, and a primary diagnosis of fungal infection in the lungs cannot be made at the bedside.

Even the pulmonary affections in those cases where the causative agent by the respective author has been classified as a blastomycete, have shown great likeness to tuberculosis, which is apparent, by way of example, from the following statement by Servet in 1922 (113): "Cliniquement, rien ne ressemble plus à la tuberculose que les blastomycoses pulmonaires."

A few accounts of autopsical findings in cases with pure bronchomoniliasis, thus without the occurrence of other micro-organisms which may be looked upon as causative agents, have been published, viz. in 1915 by Boggs and Pincoffs (15); 1921 by Mendelson (79); 1931 by Warr (130) and in 1937 by R. and J. Flinn (34). In the case related by Boggs and Pincoffs there was present also a cancer mammae sinister with abscesses, which communicated with the cavity of the chest. These authors give a description which agrees with that for an acutely or subacutely pneumonized lung. A similar picture seems to have been present in R. and J. Flinn's case, pneumonic areas in both upper lobes and left pleural effusion. — They describe furthermore in the brain yeast colonies on the surface. — Mendelson's case showed in the affected upper lobe small "tubercles", which in reality were mycotic tumours without necrosis, and stand out as very prominent masses. In the interesting case described by Warr the left lung especially was adherent to the thorax, displaying a severely congested pleura visceralis. — The patient had previously suffered from pleuritis. — The lung showed a diminution

in volume to about one-third the normal size, and was of firm consistency. It was pervaded by cavities, the largest about 4 cm in diameter, and each connected with a bronchus. These gave the lung a cheese-like appearance. Most of the cavities were smoothly lined and many were filled with a clear, jelly-like material. There was but little air-bearing in either lobe. In the right lung existed analogous changes, though less pronounced. The microscopical picture showed nothing characteristic. In a survey-work in 1927, with the title "Notes on Certain Bronchomycoses Which may Simulate Pulmonary Tuberculosis", Castellani (21) points out in pulmonary blastomycosis<sup>1</sup> the anatomico-pathological similarity to tuberculosis. The lungs may be seen to be studded with numerous white nodules. The cut-surface may show a purulent fluid, and cheesy material may be expressed from the involved region. Cavities of various sizes may be present. Histologically, central necrotic areas are seen surrounded by a zone of granulation tissue, in which giant cells are found, some of which seem to contain blastomyces.

The diagnosis of broncho-moniliasis is based on findings of yeast-like fungus not only in smears from the sputum but also by culture. Moreover, it is required that the fungus should be present in fairly large quantities, and increase in numbers with the progress of the disease. The isolation of fungi from sputum has as a rule been done by spreading in plates of a sugary medium. The simplest method seems to me, however, to be the one mentioned by Pijper (91) in 1923, who made use of the tolerance in yeast-like fungi of such a high degree of acidity, that bacteria do not endure it. He transferred the sputum from cases of broncho-moniliasis to media with pH 2, where the fungus grew, but not the bacteria existing in the sputum.

With regard to the diagnosis of broncho-moniliasis, it is most important to be able to exclude other pulmonary affections, and then particularly tuberculosis. In order to facilitate the diagnosis, Castellani has recommended intra-pulmonary inoculations into rab-

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<sup>1</sup> In Castellani's paper the *Blastomyces* are characterized in the following manner: "In the lesions large round or oval cells are present, with an exceedingly well-marked double contour and with protoplasm very granular. No mycelium occurs in the lesions. Cultures may present a fluffy appearance (due to aerial hyphae) and contain a large amount of mycelium. Asci have been described by certain authors. They do not produce gas fermentation of sugars."



bits of the isolated fungus. Tests have been carried out with complement fixation and agglutination reaction, using serum and fungal strains isolated from the cases in question. Farah (31) states in 1923 that he had succeeded in obtaining positive agglutination reaction, while in 1929 Grossi and Balog (40), as well as Hoffstadt and Lingenfelter (47), noted that the fungus cells showed spontaneous agglutination. Complement deviation was obtained in 1923 by Farah (31) and Steinfield (118), and in 1929 by Hoffstadt and Lingenfelter (47), and also in the same year by Kurotchkin and Chu (62). Negative results have, however, according to several authors, been stated. Tests for using skin reactions as an aid in diagnostics have also been made with varying success, and in regard to this subject the reader is referred to Balog and Grossi's (7) investigations in 1929.

As regards the prognosis of broncho-moniliasis, the mild type may result in a spontaneous cure, while the intermediate type may heal spontaneously after a long time; although often with exacerbations, or pass into the grave form, which terminates fatally. The ordinary therapy, which according to statement seems to have had the greatest effect upon not too severe cases, is potassium iodide in large doses. Furthermore, autovaccin is also by some authors said to have exercised a certain amount of influence, but this is denied by many.

As has been stated in the preceding, tuberculosis has been most important as being capable of exclusion in diagnosing broncho-moniliasis. In several instances yeast-like fungus has been met with in sputum together with tubercle bacilli: In 1920 Castellani, Douglas, and Thomson (24); 1921 Macfie and Ingram (72), Mendelson (79); 1923 Joeke and Simpson (50), Pijper (91); 1926 Kotkis, Washowiak, and Fleisher (58); 1929 Craig (27), Maret (75); 1931 Warr (130); and in 1937 R. and J. Flinn (34). As a rule, the fungus was then looked upon as a secondary invader to Koch's bacillus, while one or another considered they had reason to assume the contrary, Pijper (91), R. and J. Flinn (34). In his paper in 1929 on yeast-like fungus forms in chronic lung infections Maret tells us, that in his systematic differential examinations of sputa from 304 patients, he had found 62 to be negative both to tubercle bacillus and fungus infection; of the remaining 242 patients, 6.61 per cent were infected with tubercle bacilli only, 27.27 per cent

with mixed tubercle bacilli and fungus of yeast-type, and 65.11 per cent only with the latter. Marett emphasizes the importance of the fungi so strongly, that he says "that in cases in which fungi are not present the patient is usually not so much a sufferer as a carrier of tubercle". Kristenson (60) who in 1940 examined sputa from patients suffering from pulmonary tuberculosis (104 cases) and from patients without any clinical signs of actual tuberculosis (174 cases) found a yeast-like fungus in 2 per cent of the first group and in 5 per cent of the latter.

Several animal experimental investigations have been carried out for the purpose of ascertaining the pathogenity of yeast-like fungus strains isolated from sputum from cases of pulmonary affections. Most suitable as test-animals seem to have been rabbits, rats, and mice, while monkeys and guinea-pigs have sometimes proved susceptible, but dogs not at all. The results diverge to a high degree. Pijper (91), who in 1923 carried out tests with yeast-like fungus strains from cases of broncho-moniliasis, found that a quantity of 2,000 million fungus cells injected intravenously into a rabbit with great certainty killed the same within 24 hours, and after about one week, if half the dosis was used. Smaller doses occasionally caused death, but usually the animals remained alive for weeks, showing very marked emaciation and loss of power in their hind limbs. If such an animal died after some weeks the fungus could be found in sections of the organs. The most remarkable thing in such cases was *the absence of marked inflammatory changes* in such organs. It looked more as if the fungus had been quietly growing in the organs, than that a real parasite had gained access. — Similar effects, Pijper points out, have been recorded after subcutaneous injections of blastomyces by several authors. — In his inoculation tests into rabbits Pijper obtained a high agglutinative titre and very marked powers of complement fixation, but if in immunisation killed fungus was used, the result was negative, no matter how often and how large doses were used. Such results were also, according to Pijper, seen by Magalhaes. As a general rule such large doses seem to have been used, that the animals in the case of intravenous and intrapulmonary inoculation died within a short period.

After intravenous inoculation of yeast-like fungus strains isolated from broncho-moniliasis into rabbits mycotic metastases have generally been obtained in most of the organs in the form of white,

raised nodules the size of about a pin's head. Redaelli (97) in 1924 obtained always such in the heart muscle, the spleen, the suprarenals, and the abdominal lymphatic glands. According to Redaelli the fungus elements, being introduced into the circulation, either act as an embolus, and as such stay in the small vessels, obstructing them, or they form a small thrombus in the medium sized vessels, and from that fungus cells can develop and penetrate through the walls of the vessels into the surrounding tissues. The nodules consist in the centre of cells of chiefly haematogenic and lymphogenic origin, and in the peripheries of histogenic origin. Apart from the formation of nodules he also mentions what he calls distant reaction in the organs, degenerative changes, especially in the heart and liver. Besides fatty degeneration he observed zones of real necroses in the liver. The nodules may, especially in the lungs, after both intravenous and intrapulmonary inoculation of the fungus in question, be manifestly reminiscent of tubercles. They consist then of masses of small, white cells and polymorphonuclear leucocytes which decrease in numbers peripherally, and are surrounded by a ring of epithelioid cells, sometimes together with a few large, multinucleated giant cells, and also now and then foci may show caseous formation in the centre. Nodules resembling tubercles were noted even by other investigators, e. g. by Grossi and Balog (40) in 1929 (intrapulmonary inoculation into rats). Stokes, Kiser, and Smith (120), who in 1930 *inter alia* inoculated rats subcutaneously and intraperitoneally with fungus from broncho-moniliasis, obtained histological changes which fully agreed with such as occur after inoculation of tubercle bacilli. The last-mentioned authors point out that the only difference between the changes obtained and tuberculous ones was, that they were able in the foci to prove fungus elements, but no tubercle bacilli. Magrou (74) relates in 1919, that he had inoculated rabbits intravenously with a yeast-like fungus from a case of pulmonary affection, and in one rabbit, which died after 5 days, found in the kidneys inflammatory nodules of polynuclear cells, and in the centre a felting of mycelial filaments surrounded by a ring of acid-fast, claviform formations.

Lim, Kurotchkin, and Wu (70) inoculated in 1930 some rabbits intratracheally with a yeast-like fungus strain from broncho-moniliasis, but without result. It was not until after intravenous inocula-

tion of chaulmoogra-oil<sup>1</sup>, which destroyed the parenchyma in the lungs, that the fungus cells were able to grow. In various inoculation tests into monkeys with a yeast-like fungus strain from a fatally terminating case of broncho-moniliasis, Reimann and Kurotchkin (104) in 1931 obtained a negative result, although for the destruction of the lung-parenchyma pulverised glass, chaulmoogra-oil, etc. had been used.

Many other animal experimental investigations have been carried out with yeast-fungus forms from cases of pulmonary affections, which nevertheless would take far too much space to record, for which reason the quotations must serve more as illustrating examples of the results of investigations in question, than an exhaustive description.

Although, as is seen from the preceding statements, the fungus in question has many times been met with in tuberculous material containing Koch's bacilli, or in changes which are strongly reminiscent of such as occur in tuberculosis, and also that inoculation into animals many times has caused changes that agree with tuberculous ones, *the fungus has not been brought into any connection whatsoever with tubercle bacilli*, although this is extremely near.

The first to emphasize a real relation between a fungus of yeast-oidium type and Koch's bacillus, was Reenstierna who, through keen observations proved that the fungus in question was able to produce acid-fast rods (98—103). His way to this conception passed via observations of the causative agent of leprosy.

Thus, in February, 1912, he isolated a mycotic non-acid-fast micro-organism from the blood of a patient suffering from nodular leprosy in the acute stage. Growing in a special fluid medium, it formed, besides solitary large prickly cells, chiefly long chains whose links partly resembled small yeasts (Pl. III, fig. 1) and, furthermore, were able to acquire acid-fastness, thus resembling Hansen's bacilli (Pl. III, fig. 2). His cultural and animal studies convinced him that the micro-organism in question was the fungus that causes leprosy, and of which the bacillus of Hansen is merely a part.

The bacillus of Hansen and that of Koch being as like each other

<sup>1</sup> This substance causes namely, as Frazier and Chen (38) have shown in 1930, extensive lung damage when injected intravenously into rabbits.

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as twins, Reenstierna concluded that a "mother-fungus" of the latter must also exist, and that the same should also be yeast-like.

He then began, in June 1912, to examine different tuberculous material and was able to establish the occurrence, in sputa and uretero-catheterized urines, of a fungus of yeast-oidium type which was isolated and studied in *single cell* cultures. In October of the same year he made extremely interesting discoveries: In two single cell cultures (glycerin boullion) of the fungus — originating from the sputum of a patient suffering from pulmonary tuberculosis — there had *appeared* in between the non-acid-fast fungus cells, acid-fast rods which morphologically and tinctorially were indistinguishable from the bacillus of Koch. Moreover, as the plates in one of Reenstierna's papers show (103) — also reproduced in a paper by Gullberg (41) — the rods were disposed in small groups, the size and shape of which were, in parts, those of an ovoid fungus cell. Finally, fungus cells in a state of disintegration were seen, leaving behind as a remnant different bacterial elements, i. e. the finest, almost invisible powder<sup>1</sup>, cocciform organisms, diphtheroids, as well as acid-fast rods of tubercle bacillus type. Briefly, the origin of the acid-fast rods had been proved to be the fungus. Inoculations into guinea-pigs with cultures of the fungus did not result in any tuberculous changes of the inguinal glands and internal organs. Some of the animals, however, died *cachectic*. Reenstierna himself thinks that the acid-fast rods in question are to be considered as merely pre-stages of Koch's bacillus.

Reenstierna's observations were confirmed and extended by Gullberg (41) in the years 1933—1935. Thus, in 1933, he isolated a non-acid-fast fungus of yeast-oidium type from the sputa of two patients suffering from pulmonary tuberculosis, and also from the blood of a patient with high fever and erythema nodosum in his history. These three strains were studied in *single cell* cultures.

In glycerin boullion culture of two of the strains (one of them originating from the sputum and the other from the blood) there appeared, more than a year after their isolation, acid-fast rods of Koch's type. In the smears Gullberg found inter alia fungus cells, partly in granular disintegration, and in or around the destroyed

<sup>1</sup> Reenstierna draws special attention to this powder, according to him no doubt filterable, passing "les bougies et le placenta (hérité de la tuberculose; . . .) . . ."

### Working Hypothesis.

The theory of erythropoiesis now to be presented was suggested by my observations in circulating blood. The stalk-like processes on the edge of the erythrocyte disk seen so often in poikilocytosis are assumed to be real stalks by which the erythrocytes were once attached to the capillary wall. Figure 1 is a schematic representation of the formation of poikilocytes as I presume it to take place (11).

As seen from the figure, stalkless blood corpuscles would be formed when the capillary wall is extremely thin, as in normal circumstances. A thickened wall, or at any rate an abnormally long distance between the mother cell outside the vessel and the erythrocyte inside the vessel would require a bridge between them. The adherence of this link to the cell when it is detached would explain the stalk-like process of the poikilocyte. The process would thus be a malformation which reveals something of the mechanism of erythropoiesis.

This interpretation of the processes leads to a new interpretation both of the denucleation of blood corpuscles and of their manner of passage through the vascular wall.

By fitting in well-known morphologic facts with this working hypothesis, I shall now attempt to answer the question in the title of this article.

### Poikilocytosis.

Irregularly shaped erythrocytes of all kinds are given the common name of poikilocytes. They may have the shape of pears, bullets, anvils, molars or other objects (4). Most hematologists believe that poikilocytes are formed secondarily in circulating blood under the influence of non-isotonic plasma. It is presumed that blood corpuscles of these irregular shapes are of inferior quality from the start (Ehrlich, Naegeli) (4). Schulten (12) stated that poikilocytes were artefacts produced when making the smears.

Many attempts have been made to explain poikilocytes. Ebbecke (13) considered that they were forerunners of spherocytes. Schultz and Buding (14) observed budding off of protoplasm from megaloblasts in a case of pernicious anemia, and believed that this explained the development of poikilocytes. Shortly after-

and who shortly after birth had been B. C. G.-vaccinated, Gullberg found in smears from the empyemic pus yeast-like fungus cells and acid-fast rods. The latter, but not the former, were obtained in culture where they grew as Koch's bacilli and, inoculated into guinea-pigs, caused tuberculosis.

Plá y Armengol, 1931 (94) saw yeast-like elements in a 53 days old tubercle bacillus culture. Similar ones seem also to have been observed by Minchin according to Plá y Armengol, who states in his paper "... die von Reenstierna, von uns und von Minchin beobachteten hefeförmigen Formen".

Hollström, 1942, (48) in a 20 days old glycerin bouillon culture of a peculiar tubercle bacillus strain (capable of producing non-acid-fast, diphtheroid rods) was able to establish the appearance of yeast-like fungus cells, some of which contained acid-fast elements.

It is of great interest that in 1941 Schaumann (109), and also Schaumann and Hallberg (110), have been able to establish in organs from cases of Lymphogranulomatosis benigna (Schaumann's disease) the occurrence of fungus cells, which they assert as being quite similar to those described by Reenstierna in 1912, and, furthermore, that the authors in the organs in question, apart from such fungus cells, have also found acid-fast rods with the same morphological and tinctorial properties as those of Koch's bacillus. These findings are especially interesting in view of the fact that L. B. is by Schaumann regarded as being a disease of a tuberculous nature, though it does not seem to cause positive tuberculin reaction. The findings of acid-fast rods and fungus cells made have been enabled, or at least largely facilitated, by Hallberg's new method of staining tubercle bacilli (42). For with this method of staining a good many fungus elements prove to be acid-fast and the tubercle bacilli look larger and more distinct and are easier to find.

In this connexion a disease in cattle, which in American literature passes under the name of "skin lesions", is of interest. It is characterized by cutaneous and subcutaneous resistances, generally localised to the extremities, the neck and the belly, which display microscopically a picture that has great similarity to, and in many cases agrees with, genuine skin tuberculosis. They contain almost constantly acid-fast rods, which often to a high degree have the appearance of tubercle bacilli. They have been found very difficult to cultivate, and on inoculating into animals have not proved to be pathogenic,

nor have they called forth positive tuberculin reaction, which is mostly found in animals with skin lesions.

But it is of special interest that Hedström (44), as the first, in 1941, succeeded in isolating a yeast-like fungus from these skin lesions and corresponding lymph glands, which in appearance, according to the description given, seems to agree with that described by Reenstierna. Hedström has been able to cause with this fungus on inoculation into cattle changes of tissue of the same kind as in "skin lesions", and to a large extent also tuberculin allergy.

Even another fungus than the one of yeast-oidium type has been brought into direct connexion with Koch's bacillus. Dostal (39) in 1913 has namely by experimental research considered himself able to prove the origin of an aspergillus from tubercle bacilli via non-acid-fast, coccus-like micro-organisms. Single cell culture of the aspergillus in question caused, on inoculation into one guinea-pig, tuberculous changes containing typical Koch's bacilli. An NaCl-emulsion of crushed organs from this animal caused, on inoculation into two guinea-pigs, in its turn tuberculosis. Unfortunately, no pictorial material of Dostal's meticulously described tests is available.

According to Weissfeiler (131), Enderlein, too, is reputed to have obtained a transition of tubercle bacilli in aspergillus and also to have advanced a theory in which he vindicates the opinion that the bacillus of Koch originates from an aspergillus. Weissfeiler does, however, not render an account of those findings upon which Enderlein is basing his conception.

Already in 1823, one year after the discovery of the tubercle bacillus, Babes and Cornil (4) as well as in 1824 Petrone (23) observed atypical tubercle bacilli, ramified forms, as well as such with filament and spadix formations, which induced the last-mentioned to assume a connexion between Koch's bacillus and groups of organisms that are on a higher level than the bacteria. Since that time a great number of authors have emphasized the pleomorphism of the tubercle bacillus which is evident from the monographs by Kahn (51), Roman (105), Hedvall (46), Weissfeiler (131), and others. Nevertheless some few investigators adhere to the monomorphism of Koch's bacillus, e.g. Lange (65).

Several investigators have assumed a kinship between the



tubercle bacillus and the actinomycetes, or organisms closely akin to them. — An expression for the kinship between Koch's bacillus and fungoid organisms is the term *Mycobacterium tuberculosis* introduced by Lehmann and Neumann (132) in 1896. — Dalous (28) in a work of the year 1901 includes the tubercle bacillus amongst these fungi, and Lieske (69), in his "Morphologie und Biologie der Strahlenpilze", 1921, emphasizes that Koch's bacillus is so closely related to the actinomycetes that it is impossible to draw a distinct line of demarcation between these two groups of organisms. In 1930 Karwacki (52) and Kedrowsky (53); in 1931 Fejgin (32) and Møllgaard (83); and in 1935 Weissfeiler (131) observed the development of typical actinomycetes from tubercle bacilli; and in 1930 Karwacki (52); in 1931 Møllgaard (84), Triuss and Politowa (127); and in 1936 Kedrowsky (54) obtained tuberculous changes in inoculation into animals. Weissfeiler (131) draws from these tests the conclusion that the tubercle bacillus is a typical actinomycete, which in its parasitic form occurs mostly as an acid-fast rod.

The actinomycetes do not manifest kinship only with *Mycobacterium tuberculosis* but also with the leprosy bacillus and other acid-fast bacteria, some of which occur as saprophytes in nature. They have by Lehmann and Neumann (67) been placed together into the group *Mycobacterium*. It has often been found very difficult to distinguish the so-called acid-fast saprophytes from Koch's bacillus. Some of them grow on artificial media more quickly than Koch's bacillus, and, as Schlossberger and Pfannenstiel (111) have shown in 1920, both in living-room temperature and at a temperature of 50°—55° C, in which respect they agree with *Mycobacterium tuberculosis*, typus *Galinaceus*. Others have been found uncultivable. The acid-fastness of some species has been described as less pronounced, but one can also in the tubercle bacillus find great variability in this respect. Nor can the two groups of Bacteria be safely separated in regard to form. In inoculation into animals of the acid-fast bacteria occurring in nature, their pathogenity has been very varying. Some of them have been able to cause changes in the test animals, which fully agree with those obtained in inoculation of tubercle bacilli, which has been proved inter alia by Moeller (82) in 1899. On the other hand, tubercle bacillus strains have been observed with considerable variations in their pathogenity, e. g. as Pinner (93) has shown in 1932. It is highly interesting that in 1921 Kolle, Schloss-

berger, and Pfannenstiel (57) were able, for eight separate saprophytic, acid-fast strains to show by repeated guinea-pig passages increasing virulence, which agree fully with those caused by genuine tubercle bacilli. Similar observations have, according to Lehmann and Neumann (67) in 1925, also been made by Sanfelice (106) and Schröder (112). There is no certain serological reaction to distinguish the different acid-fast micro-organisms.

The opinion has been advanced that the tubercle bacilli are the parasitic form of acid-fast saprophytes growing "wild" in nature. Perhaps a way leads from human-bovine via avian and cold-blooded types of tubercle bacilli down towards the acid-fast saprophytes in nature? But this must, according to Mellon (78), still be regarded as a theory, for which evidence is lacking.

The location of the tubercle bacilli in the fungoid-bacterial system is still unclear. Castellani (20) has classified them amongst the Fungi Imperfecti, but other systematicians amongst the bacteria. Although mycobacterium tuberculosis generally occurs in Bacterial form, it may, occasionally, show mycotic qualities, which, as stated in the preceding, has induced several investigators to assume kinship between the tubercle bacillus and higher micro-organisms. This old conception is to be found in a modernized form in Pribram's (96) statement in 1927, that "Bacterium tuberculosis itself is a transitional form, not between different bacteria, but between the whole genus Bacterium and the genus Hyphomyces (fungi imperfecti)".

# OWN INVESTIGATIONS.

## CHAPTER II.

### The Cases from which the Fungus Strains have been isolated.

Out of the five fungus strains with which I have carried out my research, three have been isolated from gastric lavage from patients who, for the purpose of ascertaining possible pulmonary tuberculosis, had been sent to the Central Sanatorium at Sandtråsk (Physician in Chief: Dr. B. Carstensen). The two remaining strains have been obtained from the sputum of patients with clinical diagnosis of pulmonary tuberculosis, who were being treated at the Central Sanatorium at Upsala (then Physician in Chief: Dr. A. Kristenson). The histories of their diseases are briefly related here.

**Case I.** S. P., a woman 40 years old. In the year 1937 insidious trouble in the form of aching in the finger-, hand-, and knee-joints, as well as sometimes sensitiveness to pressure over the finger-joints. In the winter of 1938—39 she was troubled with cough with yellowish or white expectorations, but never mingled with blood, especially in the mornings. On examination at the Central Sanatorium at Sandtråsk, in March, 1939, the following diagnosis was made: Positive tuberculin reaction. B.S.R. 48 mm/1 hour. X-ray film of the lungs shows within the right ScI-I<sub>1</sub> and in the left ScI irregular spottiness and stripiness, probably fibrous remnants from an earlier process, and at the forward edge of the right C<sub>I</sub> a small calcination. Thus the X-ray examination did not supply any certain criterion for an *active* tuberculous process. The patient was taken into the same sanatorium in July, 1939. From the income status is ascertained: Pale, lean woman, grown old before her time, with habitus astenicus and typical sideropenial appearance. Heberden nodes. Indeterminate "rheumatic" ache in the joints, as well as here and there in other parts of the body. Gastro-intestinal disturbances. B.S.R. 36 mm/1 hour. No fever. Stethoscopically some unclear vesicular respiration can be distinguished over the apices of the lungs, but otherwise nothing remarkable. No sensitiveness to pressure over the finger-joints. No roentgenological change of the lung-picture since the last examination. During the fifty days' stay at the hospital the sputum has been examined seven times, no acid-fast bacilli being found on any one occasion. A cultivation on Hohn-



tervals. She has had several small haemoptyses but never any tubercle bacilli in her expectorations, and rarely fever. B.S.R. has as a rule kept between 10—20 mm/1 hour. Tuberculin positive. X-ray films of the lungs in 1933 show enlarged and congested hilus shadows and minor calcinations in the parenchyma. In the right ScI-I<sub>1</sub> cloudy confluent spots, which gradually congested and grew better delimited. The picture then mainly unchanged until 1937, when a floccula appears in the left ScI and a dissemination of congestions takes place within the right I<sub>1</sub>-I<sub>4</sub>. The state of her lungs is then on the whole unchanged until the month of May, 1939, when in an X-ray film of the lungs one can see distinct progress, in that laterally to the right C<sub>II</sub> there is a cavity(?) the size of a plum, which afterwards in a film in August of the same year shows a distinct diminution, and in films in September and November of the same year cannot be noted with certainty. The condition of the lungs otherwise unchanged. In October, 1940, the changes show strong retrogression. A streak marks the lower limit of the right upper lobe, which is drawn up to C<sub>II</sub> and in a film in February to C<sub>I</sub>. Pneumothorax started on the right side in February, 1942, and has since then been maintained. The lung collapsed rather well downwardly, but was upwardly distended by broad adherences. After burning off these the whole lung shows very good collaps. No cavity can be observed there. The condition on the left side is unchanged. In September, 1939, there was found in the sputum a *fungus of yeast-oidium type*, which was pure-cultivated on ordinary agar. In 1942 it is still present in the sputum fairly plentifully, but no acid-fast rods have ever been observed there. On inoculating into guinea-pig in 1939 with sputum a negative result was obtained.

Clinical diagnosis: *Tbc. pulm.*

Case IV. A. L., a man 41 years of age. By reason of pulmonary tuberculosis in his family an examination of his lungs was made at the Central Dispensary at Luleå in June, 1939. In the film are then seen within both ScI in parts of more recent date, in parts softer, in parts confluent, apparently calcareous spots (the film, however, not perfectly marked throughout). In order to ascertain whether he was a carrier of tubercle bacilli, the patient was in June of the same year taken into the Central Sanatorium at Sandträsk. In an X-ray film of the lungs there appear then materially only remnants of an earlier process. Nothing in the picture speaks for activity. B.S.R. 5 mm/1 hour. In cultivation on Hohn-medium from gastric lavage treated with sulphuric acid a *fungus of yeast-oidium type* was obtained in pure culture. No acid-fast rods. Inoculation into guinea-pig gave a negative result. In controlling the lungs in January, 1940, no changes of the lung-picture visible.

Clinical diagnosis: *Tbc. pulm. peracta?*

Case V. V. S., a 39 years old woman. Under treatment for tuberculosis of both lungs at the Central Sanatorium in Upsala from 2nd January, 1939, until 8th November, 1939, when she died. During the whole time the patient had tubercle bacilli in the sputum as well as a *fungus of yeast-oidium type*, which was pure-cultivated on ordinary agar.

Anatomo-pathological diagnosis: *Tbc. pulm. amb. cum caverna multiplex pulm. dexter + Laryngitis et Tracheobronchitis tbc. + Tuberculosis intestini + Steatosis hepatis + Amyloidosis lienis + Degeneratio parenchymatosum organorum.*

In sections from the tuberculous lung foci plenty of tubercle bacilli are seen and rather sparse, yeast-like fungus elements, solitary and in minor groups.

Of the five cases described three (I, II, and IV) were observed for suspected pulmonary tuberculosis, showing roentgenological changes of the lungs but no proved active process. Case III has always been looked upon as one with lung tuberculosis, though no tubercle bacilli have at any time been found in the patient's sputum. Case V has from the beginning been a serious case of progressing lung tuberculosis with tubercle bacilli in the sputum. *The fungus isolated from the five patients was of the same type as Reenstierna's of 1912.*

### CHAPTER III.

#### Isolation of the Fungus Strains and Reproduction of Single Cell Culture.

The fungus strains have been denoted by the same numbers as the cases from which they originate. Nos. I, II, and IV (from gastric lavage) were isolated on Hohn-medium, Nos. III and V on ordinary agar. Thereafter 10 spreading passages on agar plates were carried out, each time with re-inoculation from a single colony. Then the five strains were transferred into slanted agar tubes and re-inoculated on the average once every month. Having reached their 10th generation, about one year after their isolation, the fungus strains were used for the preparation of *single cell* cultures.

For the purpose of obtaining a single cell culture a Chambers' micromanipulator with inverted microscope was used. From the research material, fresh fungus cultures on agar, were emulsioned smaller quantities, less than  $\frac{1}{2}$  normal loop in 100 cc of a physiological NaCl-solution, after which drops of the same were transferred to the slide of the micromanipulator and examined. When a drop containing only *one* fungus cell was met with, this was transferred to an agar plate, where a *single* colony then developed. The techni-

cal process is analogous with that employed by for example Kahn (51). The latter worked, however, with a hanging-drop, while I, on account of the microscope used, had the advantage of a lying drop. On transferring to or respectively removing, from the slide, capillary tubes were used, which had been protracted automatically by means of a Du Bois needle-pulling machine, and then bent over a micro-flame in a suitable manner. The apparatus used is described in detail in Mc Clung's Microscopical Technique (77).

All parts of the apparatus which might come into contact with the fungus emulsion were sterilised immediately prior to the commencement of the tests by boiling for 1 hour. The slides were dipped also in Spir. conc. and fired. No other growth than the fungus in question has ever been obtained.

The *single cell cultures* thus obtained were then cultivated for 10 generations on ordinary slanted agar, before cultivation on media suitable for tubercle bacilli or animal tests were carried out.

## CHAPTER IV.

### Cultural, Tinctorial, and Biochemical Properties of the Fungus.

At a temperature of  $37^{\circ}\text{C}$  the fungus grows on the surface of ordinary agar or glycerin agar within a period of 24 hours to round, slightly arched, white colonies the size of a pin's head, with moist surface (S-form). The microscopic examination shows that some of the colonies consist only of round and ovoid cells (as a rule  $4-6\ \mu$  in diameter), and furthermore others of elongated ones, which grow out from the border, thus forming mycelial branches (Pl. I and II). After only a few days' growth the size of the fungus cells may vary in a high degree. The mycelial colonies occur more often inside the agar (Pl. II). At living-room temperature fairly good growth is obtained, though it proceeds more slowly than at  $37^{\circ}\text{C}$ . On solid substrata containing sugar, especially maltose agar, the fungus grows rapidly and luxuriantly, forming a cream-coloured mat (Pl. XXII, fig. 2), which, when the culture has become 2 to 3 weeks old, may show a puckered or relief-like surface (R-form). A dissociation in R-form may, however, also now and then occur immediately (Pl.

XXII, fig. 3). On Hohn- and Löwenstein-media the fungus grows much more slowly than on the other substrata mentioned, and may then present a picture which displays great similarity to that occurring in bovine tubercle bacilli culture.

In glycerin bouillon, too, growth is obtained in 24 hours. Generally, the fungus grows in the deeper layers of the medium as cloudy, stringy masses. The masses of fungus can, however, grow along the sides of the flask and out on the surface of the glycerin bouillon, forming a pellicle of finely meshed network, from which long cell conglomerations may suspend like rosaries or garlands. On merely lightly shaking the flask, the superficial layer of fungus drops down to the bottom. The fungus may sometimes show a granular growth on the bottom of the flask, making the medium turbid. It consists then merely of round and ovoid cells. The degree of acidity of the medium seems to play a small part for the growth of the fungus, but a poorer growth is obtained on a strongly basic medium than on a strongly acid one, and a moderate degree of acidity seems to be more favourable. In varying the glycerin content from 3 to 15 per cent no difference in the manner of growth, or the rapidity of growth, could be noticed. Nor did any difference become manifest when cultivating under aerobian or anaerobian conditions. In none of the different fungus cultures investigated could sexual reproductions be demonstrated.

In 24 hours' cultures the fungus cells are generally acid-fast on staining according to Hallberg (42) as well as gram-positive, but some of the cells lose within the next few days completely, or partially, their acid-fastness, so that for example in a 7 days' culture one may find all transitions between purely acid-fast and non-acid-fast ones. The gram-positiveness, too, decreases gradually, as does also, though more slowly, the general stainableness. On staining according to Ziehl-Neelsen, without counter-staining, the fungus is as a rule in fresh cultures faintly rose-coloured, with a frequently excentrically recumbent, unevenly outlined, acid-fast "nucleus". Methylene blue counterstain mostly conceals the red or rose-coloured stain (Pl. III, fig. 3), so that in only a minor number of the fungus cells the red "nucleus" is visible, and is then sometimes able to manifest itself as a faintly reddish cap (Pl. V, figs. 4—6). When staining in general, and especially older cultures, remarkably great variations of colour may be obtained. When cultivating on a blood agar



plate no haemolysis results, and in ordinary gelatine no colliquation.

Fully two years after the isolation of the fungus strains preliminary tests of their behaviour towards different species of sugar were carried out, as is shown by the subjoined table. (No test of acid formation was made.)

Strain	I	II	III	IV	V
Glucose.....	+	+	+	+	+
Galactose.....	—	—	—	—	—
Maltose.....	+	+	+	+	+
Saccharose.....	—	—	—	—	—
Laevulose.....	+	+	+	+	+

+ = development of gas                      Sugar content 0.5 %

— = no development of gas

The five strains disintegrated glucose, maltose, and laevulose, in which according to Castellani and Chalmers (23) they agree with *Monilia pinoyi*. In repeated tests about 6 months after the preliminary ones the below-mentioned carbohydrates were used in a solution of 0.5 per cent and with an admixture of Andrade's indicator (neutralized acid-fuchsin) in a concentration of 1 per cent and in a fermentation tube. — The medium in the tubes amounted to about 10 cc, and to each tube was added a normal loop of fungus from a fresh agar culture.

A. Monosaccharides

a. Pentoses

Arabinose

Xylose

Rhamnose

b. Hexoses

Glucose

Laevulose

Mannose

Galactose

B. Disaccharides

Saccharose

Maltose

Lactose

C. Trisaccharides

Raffinose

D. Polysaccharides

Starch

Inulin

Dextrin

E. Alcohols

Glycerin

Adonite

Mannite

Sorbitol

F. Glucosides

Salicin

Aesculin

In no tube was obtained gas or acid formation, although good growth had developed in all tubes, and the definite reading was not made until after 15 days.

*The fungus seems thus in the six months that had passed between the two tests to have lost its capacity to disintegrate species of sugar.* Furthermore, we do not know if the results achieved in the preliminary tests are those that would have been obtained with the cultures, if such tests had been carried out with the fungus strains immediately after their isolation.

## Appearance of Acid-fast Rods amongst the Fungus Cells.

In a glycerin bouillon culture from strain I it was possible after 35 days' growth at 37° C to discern only some solitary acid-fast, tubercle bacillus-like rods, while in another, which had been growing at 37° C for 30 days and then in living-room temperature for 32 days, a large accumulation of acid-fast, granular rods (Pl. IV, fig. 2) could be observed. In a glycerin bouillon culture of strain II were seen after 36 days at 37° C amongst the round and ovoid fungus cells faintly stained ones of which a couple contained granules, which were partly lying in diplo- or strepto-units (Pl. III, fig. 4). Furthermore, there occurred in smears taken after both 36 and 45 days rather plenty of non-acid-fast rods with acid-fast granules at the end (Pl. III, fig. 5), and accumulations where some of the rods were entirely acid-fast (Pl. III, fig. 6), and also groups of solely acid-fast, granular rods (Pl. III, fig. 8). Some of the acid-fast elements were filiform (Pl. III, fig. 7), and in one smear it seemed possible to see how an acid-fast, filiform rod issued from a fungus cell (Pl. IV, fig. 1). The long, acid-fast micro-organisms might sometimes appear as a net-work, which on Gram-staining was positive (Pl. III, fig. 11). There were also met with accumulations that could be fairly dense and consist of acid-fast elements, which, at least in their peripheries, displayed a distinct rod-shape (Pl. III, figs. 9 and 10). In two glycerin bouillon cultures of strain III there appeared acid-fast rods. In one of them were found in smears from the culture after 9 and 13 days' growth at 37° C around the fungus cells amorphous, yellowish-brown masses containing differentiated rods, partly of the same yellowish-brown colour, partly acid-fast and arched (Pl. IV, figs. 3 and 4). Furthermore, granular and semicircular elements (fig. 3) occurred, forming the outlines of light-blue stained, more or less disintegrated, fungus cells. Finally, some very large, brown, cystiform formations (fig. 4) could be ob-

served (probably swollen fungus cells in a state of disintegration). In the second culture from strain III, where the glycerin content was 15 per cent as against the usual 3 per cent, were met with after 12 days at 37° C acid-fast rods of the same appearance as Koch's bacilli, partly diffuse and partly in bundles (Pl. V, figs. 1 and 2). *In altogether five glycerin bouillon cultures from three out of the five strains originating from single cells there had thus from the fungus cells been produced acid-fast rods.* In a couple of glycerin bouillon cultures started from organs of rabbits inoculated with the fungus in question there occurred acid-fast rods (Pl. V, figs. 4—12 and Pl. VI, fig. 1). An account of these findings will be rendered in Chapter VIII.

In spite of the absence of acid-fast rods I wish to deal in this connection with a finding in a glycerin bouillon culture from strain I. In a smear from this, taken after 35 days' growth at 37° C, peculiar corpuscles, much larger than the surrounding fungus cells, were observed. With Hallberg-stain they were of a yellowish colour and plentifully provided with red prickles (Pl. V, fig. 3). As to shape and appearance, they agreed with the pine-cone-shaped formations which Hallberg — terming them briefly "cones" — had demonstrated in cultures of the fungus here concerned, in sputa of patients suffering from pulmonary tuberculosis, as well as in different tissues from Schaumann's disease (43).

## CHAPTER V.

### Inoculation of Agar Culture into Rabbits.

On account of the slight knowledge of the pathogenity and possible capacity of the fungus in question to generate in the animal organism acid-fast rods, it has from the beginning been possible to organize the animal tests only roughly. These have been carried out with varying doses of injection, intervals between inoculations, age of cultures, etc. After that, supplementary tests have been carried out. This holds good not only in regard to tests with agar cultures but also in the case of inoculation with other material. Morbid symptoms and organic changes in the animals inoculated have been mentioned briefly in connection with the tests, but described in causal statistics (p. 79) and summarised in Chapter VII. Furthermore, it has

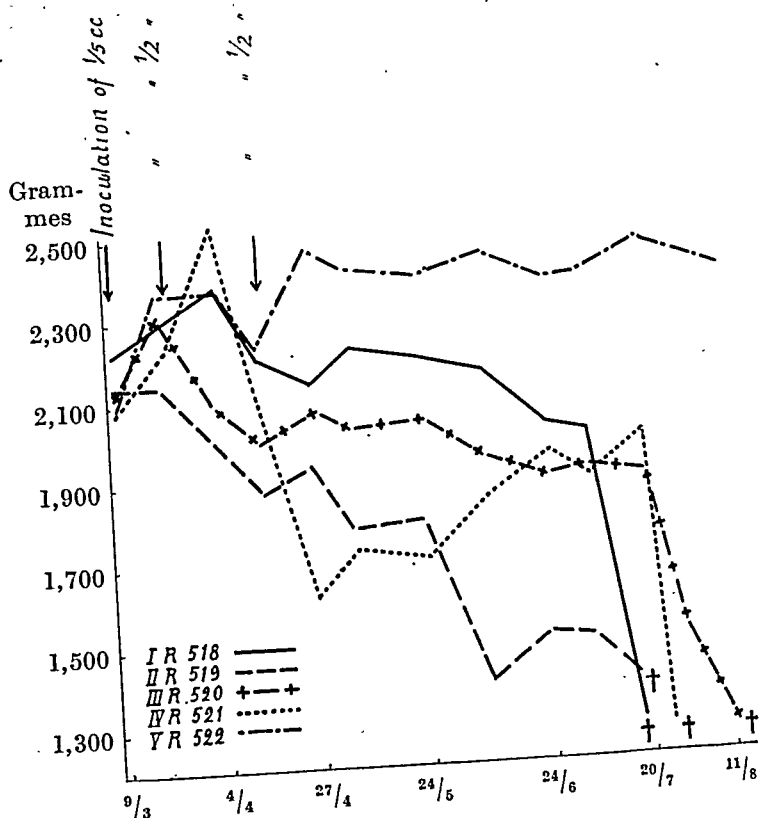
Cabot (30) reported that edema was present in about sixty-four per cent of his cases of pernicious anemia. Eppinger (31) observed thickening of the vascular walls of the spleen in pernicious anemia.

If the stalk-like processes develop as I assume, the non-nucleated blood corpuscle must be attached to the mother cell through the capillary wall long enough for the development of a real cellular membrane. During the time of maturation within the blood vessel, the blood corpuscle changes character. Its protoplasm changes from a gel to a sol while a genuine membrane is being formed. At the same time it changes in shape. When it is fully ripe, the erythrocyte breaks off and is washed away by the blood stream. It is then disk-shaped, has a tendency to aggregate and is purely acidophil on staining according to Pappenheim.

The mature, disk-shaped erythrocyte cannot pass through capillary walls. For the proerythrocyte (reticulocyte) to be able to do so, it must be of another nature. Its cloudy structure, non-specific, more or less spherical shape and tendency to basophilia indicate that it is more like other blood corpuscles. Like thrombocytes and leukocytes, therefore, it should be able to pass through the sinusoid wall. According to the hypothesis, the cell is normally connected with the mother cell throughout the process of maturation. If the proerythrocyte breaks away early, it is not certain that it is able to mature into a normal disk-shaped cell. It can become acidophil (after-ripening) but whether it can become disk-shaped is a question. Bundle-shaped proerythrocytes in circulating blood should perhaps be regarded as abortions, which immediately begin to disintegrate. During the reticulocyte crisis, there is no real increase in the total number of red cells. Not until the reticulocytosis begins to diminish is there any increase. The more mature the erythrocyte is before it loosens, the larger chance it should have of living a normal length of time.

The proerythrocyte probably acquires a surface membrane of some kind on its first contact with the blood. As the proerythrocyte is slightly larger than the mature erythrocyte, it is probable that its contents become more concentrated as it ripens. If so, a kind of negative pressure would result which may help to produce and maintain the biconcave disk shape. However, no doubt several different factors cooperate in the maintenance of the disk shape. The colloid content of the surrounding fluid obviously plays a large part. The disks soon change into spheres in fluid

Diagram 1.



In diagram 1 it is seen that four of the animals died after<sup>1</sup> 3 to four months. They manifested pronounced *cachexy*, with losses of weight between 700 and 900 gr. The spleen, which was atrophic, was examined histologically in three of the animals, and displayed then in the pulp a moderate to strong disintegration of the blood. The organs otherwise showed only minor changes. Only in one case were remnants of fungus observed. The fifth animal, R 522, was killed after about 5½ months, when it was found that no emaciation had taken place, nor any macroscopic changes in the internal organs.

### Series 3.

Series 3 was started with 5 rabbits, R 552, 538, 539, 540, 541. They were meant to be inoculated intravenously 4 times with 2

<sup>1</sup> Unless otherwise stated the time always refers to the last inoculation.

to 12 days old cultures (one of the five strains for each rabbit). The chief purpose was to try to obtain acid-fast rods in the animal organism.

Two of the rabbits died within 15 days, one, R 538, of coccidiosis, and the other, R 539, of suspected such, both having been inoculated only once. R 540 was inoculated only twice on account of severe disturbance of balance, while the remaining two rabbits were inoculated 4 times with respectively  $\frac{1}{5}$ ,  $\frac{2}{5}$ ,  $\frac{2}{5}$ , and  $\frac{1}{2}$  slanted agar culture. Since series 3 by reason of the intercurrent deaths had been thus decimated, a further 5 rabbits, R 553—557, were inoculated in the same manner.

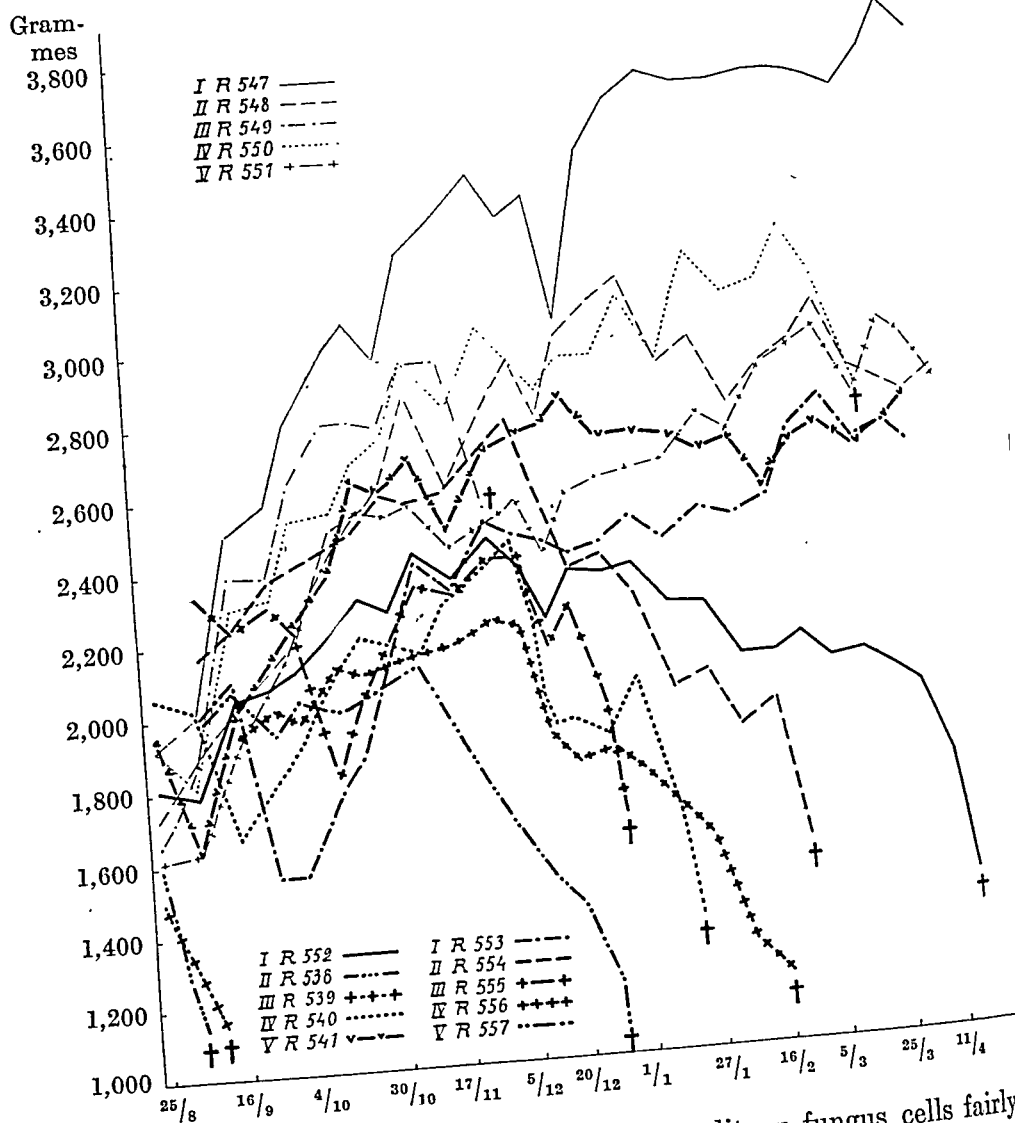
In order to ascertain at the same time whether the loss of weight manifested in four of the animals of series 2 was caused by the fungus cells as living organisms or by endotoxins, 5 rabbits, R 547—551, one for each strain of fungus, were inoculated intravenously 4 times with slanted agar cultures that had been killed by heat ( $58^{\circ}\text{C}$ — $\frac{1}{2}$  hour). — Fungus cells thus killed do not seem to be changed in size or shape, and the endotoxins, according to what Buss (16) has observed in his tests with a yeast-like fungus, will not be destroyed at a temperature of  $80^{\circ}\text{C}$  for  $\frac{1}{2}$  hour. All animals were inoculated in the course of one month.

Of the ten rabbits (see diagram 2) inoculated with live fungus culture, apart from the two previously mentioned ones, a further six died, these showing signs of disturbances in the central nervous system.

R 540, which after 9 days had shown serious disturbance of balance, after which there occurred gradually progressing paralysis of the hind part of the body, died *cachectic* within 5 months. At the autopsy the liver showed some single miliary, slightly fibrous foci, which in smears proved to contain some solitary fungus cells in a state of degeneration and "cones", but in sections only an increase in the periportal connective tissue. In the spleen was seen a faintly clear haemosiderosis. The brain displayed some ganglial changes.

In R 554 were observed after about  $2\frac{1}{2}$  months moderate paresis of the hind legs and at the same time progressing bilateral infection of the eyes. It died after a further 2 months, during which it grew very lean (from 2,790 gr. to 1,580 gr.). The lungs showed diffuse bronchopneumonias with commencing necrotic disintegration.

Diagram 2.



In smears from here were found some solitary fungus cells fairly plentifully intermingled with "cones" and gram-negative rods. The abdominal organs showed only moderate inflammatory changes, but no findings of fungus cells or bacteria were made there. In the eyes were noted purulent changes, and in the brain were found, besides perivascular round cell infiltrations, a couple of necroses with concrement-like formations.

R 555, 556, and 557 died after respectively 3, 4, and hardly 3 months, the first-mentioned with reduced flesh on the body, and the two last ones *cachectic*. R 555 and 557 showed no changes of

internal organs, apart from a moderate haemosiderosis in the spleen of R 557. R 556 showed purulent bronchitis and bronchopneumonia, which proved to contain sparse fungus cells and plenty of bacilli of proteus type. In the central nervous system of the three rabbits were noticed diffuse, minor round cell infiltrations.

R 552, which after 9 weeks showed permanent disturbance of balance, and after a further 3 months progressing paresis in the hind legs, died about 2 months later. In one lung was noticed, just below the surface, a pneumonic focus the size of a hemp seed, which contained solitary fungus cells and minor fragments of cells. Besides considerable splenic atrophy, with strong haemosiderosis, the abdominal organs were otherwise without appreciable changes. In the brain were found, apart from perivascular round cell coatings, plenty of corpuscles of the same size and appearance as those described by Wright and Craighead (133) and Levaditi, Nicolau, and Schoen (68) in a form of stable encephalitis in rabbits, and by the three last-mentioned authors termed *Encephalitozoon cuniculi*.

In R 541 and R 553 disturbance of balance occurred first after eight months. They were killed  $3\frac{1}{2}$  months later, when both were fairly well nourished. R 541 showed, besides diffuse perivascular round cell infiltrations in the brain, no appreciable organic changes, while R 553 showed moderate haemosiderosis in the spleen, a diffuse induration of connective tissue in the kidneys, and minor granulomata in the liver and brain.

Two of the rabbits inoculated with killed fungus culture died. One of them, R 549, died after 2 months of tracheitis with masses of mucus, which largely obturated the upper part of the tracheal lumen, but without macroscopic changes of internal organs, and the other, R 550, after hardly 6 months of pasteurellosis. Both were well nourished. R 551 presented after  $2\frac{1}{2}$  months an infection on one side of the gluteal region, which healed in about one month. During this time the rabbit displayed no increase in weight. The remaining animals, R 547 and R 548, survived and had not lost any weight.

*In the diagram there appears a considerable difference in the weight between the animals which died after inoculation with killed bacilli and those that died after inoculation with live cultures. The former weighed respectively 2,500 and 2,830 gr., the latter from 1,080 to 1,675 gr.*



Simultaneously with the animals in series 3 were weighed 3 non-inoculated rabbits, and these kept their weight all the time within the limits of those inoculated with killed fungus.

## CHAPTER VI.

### Inoculation of Glycerin Bouillon Culture into Rabbits.

Intravenous inoculation of glycerin bouillon cultures in varying single doses have been carried out, without the results materially differing from those that had been obtained in analogous tests with agar cultures.

#### Series 1.

Five rabbits, R 526—530, one for each fungus strain, were inoculated twice intravenously, with 7 days intervals. The cultures had previously been centrifugated, after which the glycerin bouillon was pipetted off and replaced by the same quantity of a physiological NaCl-solution.

Two of the five rabbits, R 526 and 528, were killed after nearly six months. One of them had manifested a left-side cataract and moderate obliquity of the head. Both showed reduced flesh, but at the autopsy no macroscopic changes in the internal organs. Two rabbits died intercurrently of respectively rhinitis and coccidiosis.

R 527, whose initial weight was 2,100 gr, diminished after the second inoculation greatly in weight, so that one month later it weighed only 1,250 gr. Its general condition of health was so strongly impaired that it was expected to die. After another 14 days there were noticed on the belly two subcutaneous resistances. The rabbit gradually increased in weight, so that, when it was killed after about 6 months, it weighed 1,970 gr.

At the autopsy *macroscopically tuberculosis-like changes* were established *in the lungs, liver, and spleen*. The microscopical examination of sections from the lungs showed pictures which *in no small measure were reminiscent of tuberculosis*. There was found a peculiar granulomatous process with partly caseous, necrotising granulomata the size of nearly a pea, where the granuloma cells are epithelioid and frequently mixed with giant cells in large quan-

titities. In smears, the same as in sections of the lungs, there occurred *plenty of acid-fast rods of Koch's type* (Pl. VIII, fig. 1), as well as *sparse, degenerated fungus cells* and small gram-negative, bipolar rods. The liver and spleen presented diffuse, small granulomata with giant cells but without necrosis, yet with *acid-fast rods* (Pl. VIII, figs. 2 and 3), *which on being cultivated grew in pure culture*. (For cultures of the acid-fast rods and animal tests with these, an account is rendered in Chapter IX.)

Of the afore said two subcutaneous resistances on the belly, one still remained. On examination it was found to consist of a detritical mass containing *no acid-fast bacilli* but *small gram-negative, bipolar rods*, which on cultivating in blood agar plates grew as non-haemolyzing colonies resembling dew-drops.

Such subcutaneous abscesses<sup>1</sup> containing similar non-acid-fast rods, but no acid-fast elements, occurred in 7 further rabbits, of which two had been inoculated with fungus culture 4 months earlier, and 5 were not inoculated. Furthermore, the animals that were killed two months after the appearance of abscesses, did not show any loss of flesh nor any macroscopic changes of internal organs.

There are thus no criteria for the acid-fast rods detected in the lungs, liver and spleen of R 527 being derived from the said stable infection.

## Series 2.

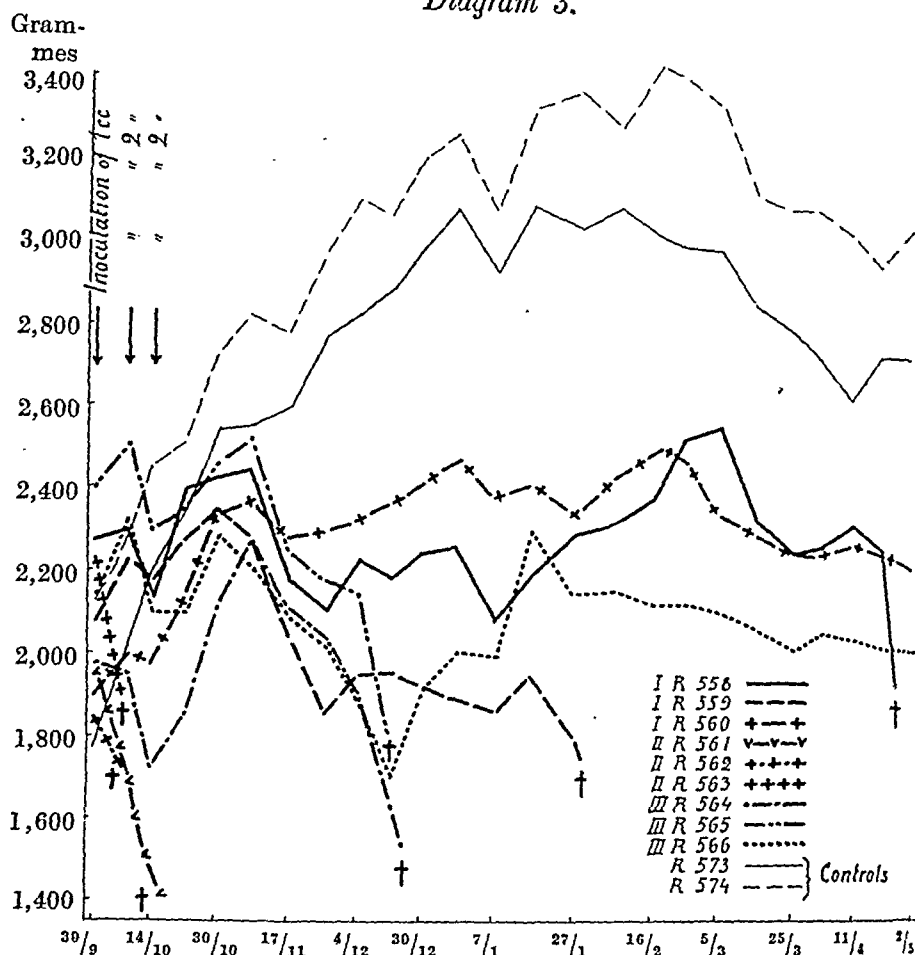
Fifteen rabbits, three for each fungus strain, were inoculated intravenously 3 times with respectively 8, 16, and 22 days' glycerin bouillon cultures of fungus. The first dosis of inoculation was 1 cc, and the 2 ensuing ones 2 cc. The changes in weight of the rabbits, as well as those of the non-inoculated ones, can be followed in diagrams 3 and 4.

Diagram 3 shows that a distinct difference in weight exists between the animals inoculated with the strains I, II, and III, and the controls.

Of the rabbits inoculated with strain I, the first one, R 559, died

<sup>1</sup> According to a statement by Dr. Hedström at the State Veterinary Bacteriological Institute in Stockholm, such subcutaneous abscesses containing gram-negative pasteurilla-like rods are very common in rabbit. In the more than 100 cases which he had the opportunity to examine he was neither able to detect acid-fast bacilli in the abscesses nor to place the abscesses in any connection with tuberculous changes (45).

Diagram 3.



*cachectic* after  $2\frac{1}{2}$  months, but without any macroscopic changes of internal organs. The second, R 558, died intercurrently after about 6 months, and the third, R 560, was killed after  $11\frac{1}{2}$  months. All of them had during their lives shown disturbances of the central nervous system, and the brain of R 560 showed the same microscopic picture as R 552 (spontaneous encephalitis), but in the others only round cell infiltrations or granulomata. The liver of R 560 showed changes that were analogous to those in the brain, though without necrosis or findings of micro-organisms. Both this animal and R 558 had eye infections, and in a vitreous body abscess from the last-mentioned were found plenty of fungus cells.

The rabbits that had been inoculated with strain II died within 17 days after the commencement of the tests, and two of them, R 562 and 563, had only been given one inoculation. In the kid-

neys of these were seen plenty of nodules that held large quantities of fungus cells. In R 561, which 3 days after the last inoculation had shown severe disturbance of balance, were discerned on microscopic examination plenty of abscesses in the brain (Pl. XII, fig. 2), and in these sparse fungus cells (Pl. VII, fig. 4). In the lungs there occurred plenty of bronchogene abscesses, in the liver abscesses, from which on cultivation there was obtained a growth of fungus, and in the kidneys chronic focal nephritis.

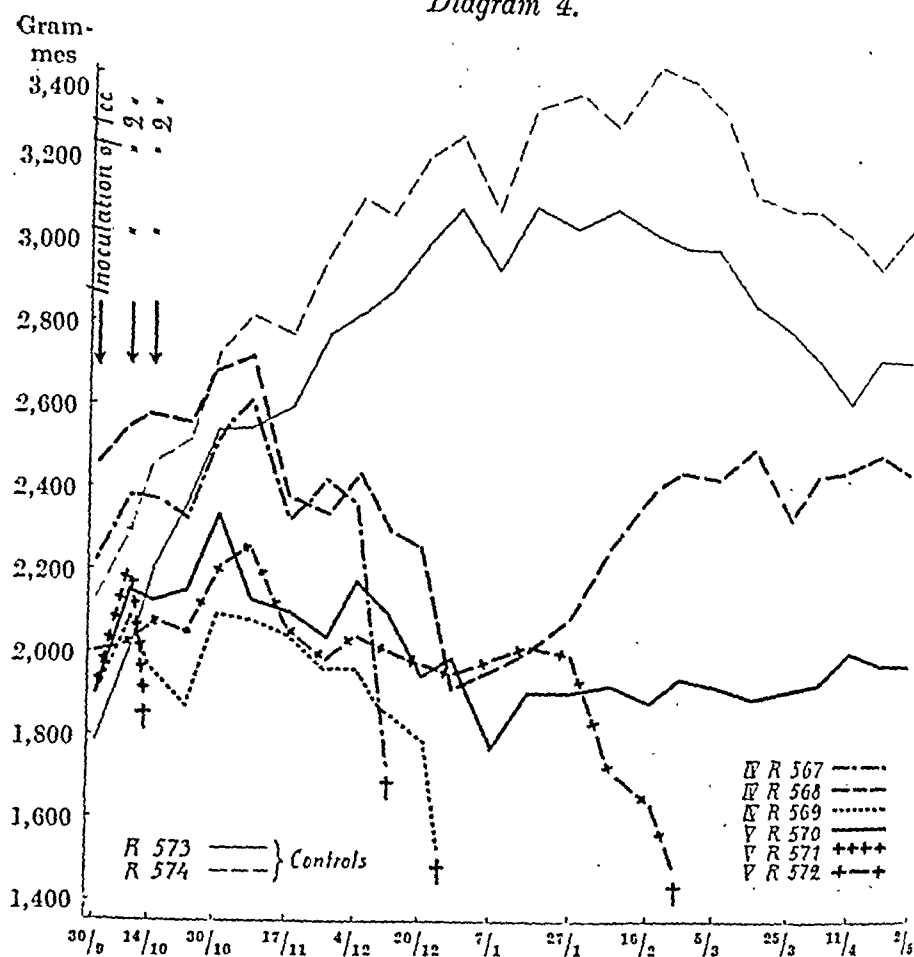
Of the rabbits inoculated with strain III, R 556 died after 2 months with paralysis of the back part of the body. It showed granulomatous changes of the lungs, liver and kidneys, as well as pronounced haemosiderosis in the spleen. In smears from the lung and kidney were found sparse fungus cells. The central nervous system showed diffuse round cell infiltrations and granulomata. R 565, which was also fully paralysed in the back part of the body, was killed after 2 months by reason of large, decubital ulcerations in the gluteal and perineal region. Its internal organs showed no appreciable changes. R 566 was killed after  $11\frac{1}{2}$  months. The internal organs were, apart from a pronounced haemosiderosis in the spleen, without any appreciable changes. Both eyes manifested endophthemia chronica and dissolution of the lens.

Diagram 4, too, shows a considerable difference in weight between the inoculated animals and the control rabbits. Nevertheless, one of the former, R 568, seems after about 4 months to increase in weight, so that it does in no very great degree differ from one of the control animals.

Two rabbits, R 567 and 569, of those inoculated with strain IV died within  $2\frac{1}{2}$  months with paresis in the hind legs and reduced weight, but without macroscopic changes in the internal organs. In R 568, which had shown a temporary loss of weight and likewise temporary paresis in the hind legs, there were found in the brain the same changes as in R 552 and 560. In its eye there was present chronic iridocyclitis, with retinal loosening, and in a subretinal abscess were found two small groups of fairly large and slightly clumsy *acid-fast rods* (cf. Pl. VII, fig. 3).

One of the animals inoculated with strain V, R 571, died after four days. There was then found in the left pleural cavity a teaspoonfull of blood-stained exudation, which in smears and in cultivation gave negative findings of micro-organisms. In the liver were

Diagram 4.



seen some miliary nodules, which contained a moderate quantity of fungus cells. R 572 died after 4 months with paralysis in the back-part of the body, double-sided eye infection, and pronounced *cachexy*. Besides bleeding from the lungs, no changes in the internal organs were found. The eyes showed vitreous body abscesses with a large amount of fungus cells, while in the brain only trifling round cell infiltrations were met with. R 570, which had presented moderate paresis in the hind legs, and hyalitis oculi bilateralis, with secondary cataract, was killed after 11½ months. The rabbit was rather emaciated and showed granulomatous foci in the lungs and liver, fibrosis of the kidneys and moderate haemosiderosis in the spleen. In the eyes was distinguished retinal loosening with an accumulation of pus and a fusion of the edge of the lens, and in the central nervous system diffuse granulomata and fresh degeneration of the pyramidal tract.

### Series 3.

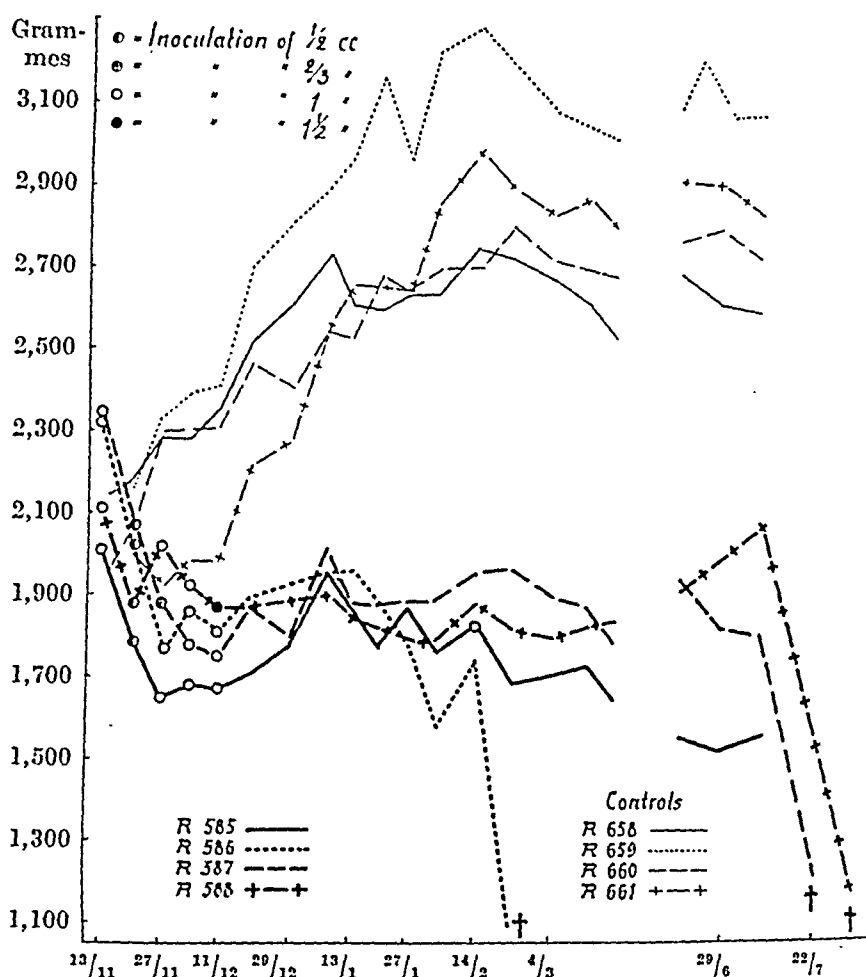
This series was started a few days after the autopsy of R 527, where some findings of acid-fast rods were made in tuberculoid changes. The aim and object here was, in doses of fungus cultures adapted to the general condition of health of the rabbits, to keep their condition down sufficiently for the capacity of the inoculated fungus cultures to bring forth acid-fast rods thus perhaps being facilitated.

As I thought that strain II displayed greater pathogenity, 6 rabbits were inoculated with this and 16 rabbits with the remaining fungus strains, 4 for each of these. In the diagrams 5—9, one for each fungus strain, the doses of inoculation can be read. As the rabbits' general condition of health can largely be judged by their changes in weight, weight curves have, for purposes of comparison, been included in the diagrams for 4 non-inoculated rabbits.

*Rabbits inoculated with strain I:* Diagram 5 shows that a distinct difference in weight exists between the inoculated animals and the control ones. R 587, 586, and 588, died after respectively  $2\frac{1}{2}$ ,  $7\frac{1}{2}$ , and 8 months, all of them with pronounced *cachexy*. R 587, which had had progressive paresis in the back part of the body, showed in the liver caseous necroses, with a fairly large quantity of fungus cells, while in the central nervous system in the sections nothing certain pathological was met with. In R 586, which had shown the same symptoms as the preceding one, was found a one-sided, strongly hydrocephalus internus, as well as malacic foci and diffuse perivascular glial infiltrations, while in the internal organs, apart from splenic atrophy and trifling changes of the kidneys, nothing appreciably pathological was noticed. In both eyes there existed diffuse changes, with large masses of fungus. R 588 died of coccidiosis, and 585, which had shown some cerebral symptoms, was killed after  $7\frac{1}{2}$  months. In the brain of the latter were found the same changes as those described for R 552.

*Rabbits inoculated with strain II:* Within 6 months all the animals had died. (See diagram 6.) R 589, which died 7 days after the first inoculation, showed in the liver and kidneys nodules the size of a pin's head, consisting of submiliary abscesses, surrounded by more or less distinct granulomatous zones. In the abscesses were found plenty of fungus cells and a few acid-fast, rather short, rods. Cultivation from

Diagram 5.



the kidney on Hohn-medium resulted in a *growth of fungus and acid-fast rods* (see Chapter VIII and Pl. VI, fig. 2). R 590, which had shown paresis in the back part of the body, died *cachectic* after hardly three months. Apart from atrophy of the spleen, with haemosiderosis, the organic changes were slight. R 591 showed after one month symptoms of disturbances of the central nervous system and died a week later, with typical signs of insufficiency of the heart. In the right chamber of the heart there was a pronounced verrucose endocarditis, the verrucose excrescences mostly consisting of large masses of fungus elements (Pl. IX, fig. 1). In the lungs were noticed minor abscesses, and in the liver several necroses (Pl. IX, fig. 2), from which in a cultivation on glycerin bouillon a *growth of fungus and acid-fast rods* was obtained (see chapter VIII). In the central nervous system occurred

### Method.

The method I have followed in the course of my investigations has differed somewhat from that of Lindahl, and I propose to give a short account of my findings.

My method is based on that of Jansen which has been modified by Wang and Harris and by Wassmann. A report by Fürst jun. has also helped me in my investigations. As only slight changes have been made in Wassmann's method, reference is made to his study for a complete account of his method.

I have, however, found it most convenient to employ the original preliminary test of Wang and Harris as Wassmann's preliminary test entailed the use of such large quantities of cerisulphate solution — up to 20 ml — that the work became tedious for this reason. More recently, Wassmann has informed me that he has been obliged to dilute the urine five times, and he urgently advises the employment of his preliminary test. With this method the fluorescence is read off by direct visual inspection in front of a quartz-mercury lamp (Philips HPW 120), and the fluorescence of the test is compared with that of a control solution in which the aneurin is not oxidized to thiochrome, and which should thus contain the non-specific fluorescent substances in the urine. I have found it convenient to work with a standard which contains 10  $\gamma$  per ml, whereas Wassmann has worked with a standard of 1  $\gamma$  per ml.

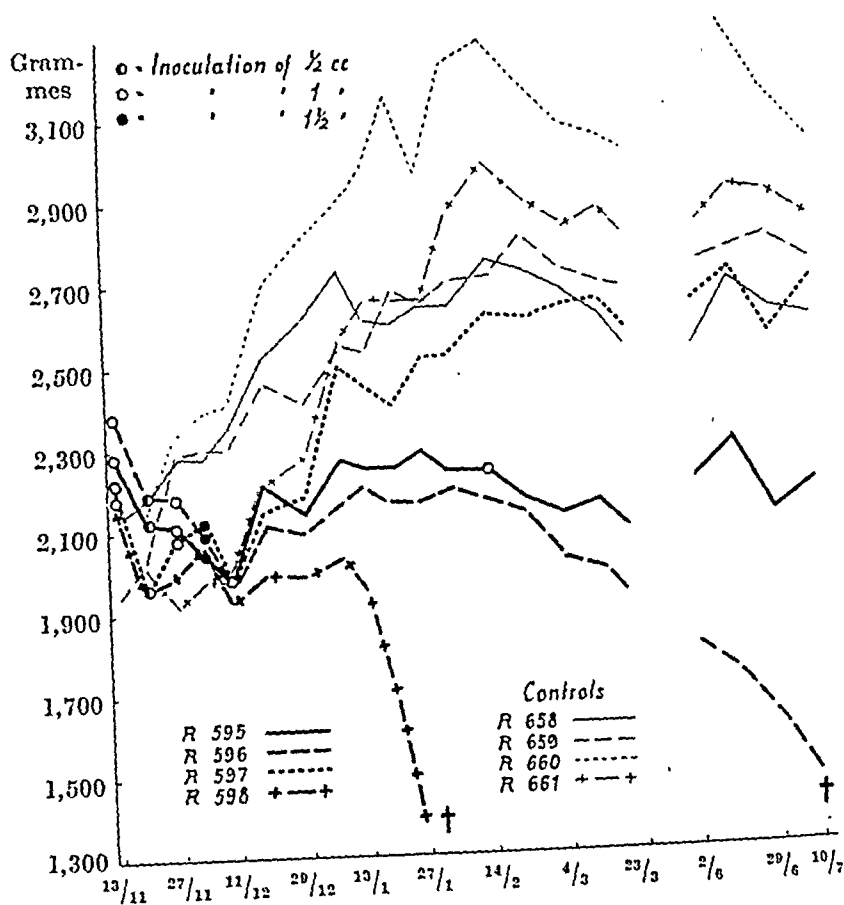
By this method and by the addition of known quantities of aneurin to the urine, I have obtained the following results (table 1). It will be seen that added aneurin has been recovered in quantities of 64 to 114 per cent. The result coincides in the main with those of Lindahl.

Table 1.

Aneurin added per 100 ml		Aneurin found	Recovery %
Urine I.	0	28	—
	7.2	34	83
	17.2	45	99
	27.2	58	91
Urine II.	0	55	—
	4.5	59	89
	14.5	72	85
	24.5	79	96
Urine III.	0	31	—
	17	44	77
	14	40	64
	7	39	114



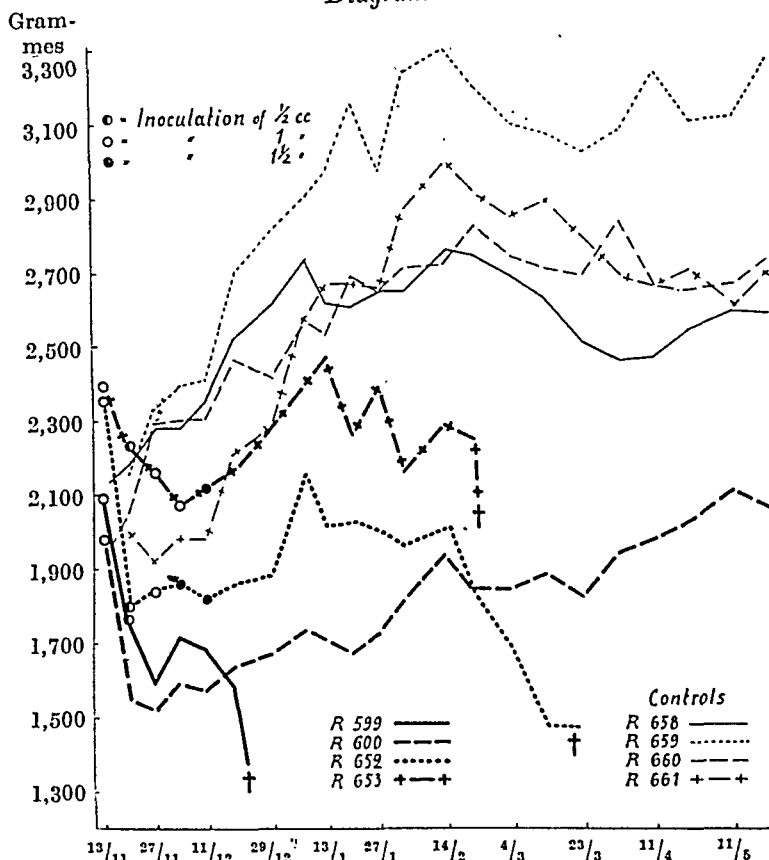
Diagram 7.



in the hind legs, died after 7 months with pronounced *cachexy*. The internal organs showed no appreciable changes. In the central nervous system was found, apart from some perivascular round cell infiltrations, a lump of exudate lying freely in the fourth ventricle, and the bulk of this lump consisted of fungus cells. R 595 and 597 were killed  $8\frac{1}{2}$  months after the commencement of the test. They showed only some uncharacteristic changes in the internal organs. All the three last-mentioned rabbits presented pronounced changes in the eyes in the form of periferal disintegration of the lens and retinal loosening, with subretinal abscesses containing plenty of fungus elements.

*Rabbits inoculated with strain IV:* In diagram 8 it is seen that there exists a difference in weight between the inoculated and the control animals. The former all display signs of injuries in the central nervous system, and on microscopic examination moderate

Diagram 8.



or fairly pronounced changes there, but without any findings of fungus cells.

R 599, which after the second inoculation proved to be severely affected, was no more inoculated. It died after one month. At the autopsy there was found a large abscess, which issued from the mediastinum and followed the large vessels up into the upper apertura thoracis, after which it passed down along the left half of the thorax and subcutaneously upon the back. The pus contained solitary fungus cells, a gram-negative, non-acid-fast actinomyces-like mycelium, and gram-negative rods. On cultivation from blood and pus there was obtained only a growth of the two last-mentioned organisms. With the exception of severe haemosiderosis in the spleen, no appreciable changes of internal organs were noticeable. R 653 died after  $2\frac{1}{2}$  months with some loss of weight. Postmortal changes

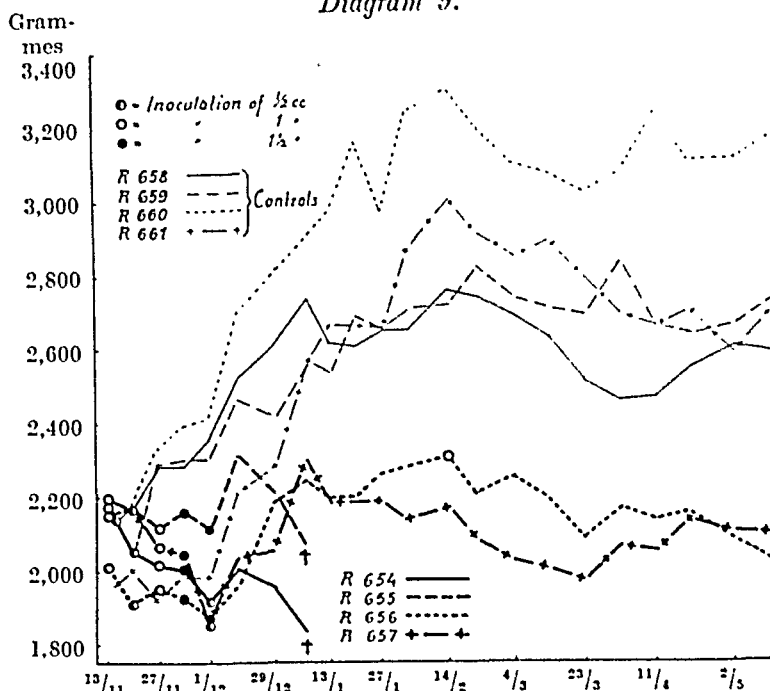
made the findings in the abdominal organs difficult of being judged, but on cultivation from the enlarged spleen no growth was obtained. One eye showed periferal disintegration of the lens. R 652 died *cachectic* after 3 months. In the liver were seen a couple of miliary necroses and in one kidney some submiliary, encapsuled abscesses, in whose centres there were lying some fungus colonies (Pl. XI, fig. 1). In the eyes there existed retinal loosening and abscesses, some of which contained large masses of fungus elements, and one of them furthermore two small groups of *acid-fast, somewhat clumsy, rods* (Pl. VII, fig. 3). R 600, which had been greatly emaciated and had shown disturbance of balance and paresis, improved gradually and increased in weight, so that, when it was killed after  $10\frac{1}{2}$  months, it did not appreciably differ from the control animals. Only moderate changes of internal organs. Both eyes showed retinal loosening and periferal necrosis, as well as disintegration of the lens. In one eye there were also met with in an abscess a couple of fungus cells with a brown self-colour. In the brain were found changes that have been described for R 552.

*Rabbits inoculated with strain V:* Diagram 9 shows a distinct difference in weight between the control animals and the inoculated ones, two of which however soon died.

R 654 and 655 died both after 3 weeks through external violence (bitten in the scrotum). Their internal organs did not display any appreciable changes. The weight of R 656 and 657 had during the greater part of their lives been stationary, but rose gradually, so that, when the animals were killed about  $8\frac{1}{2}$  months after the commencement of the tests, it did not differ so very much from that of the control animals. Besides a pronounced myocardial fibrosis in R 656, no major changes were discerned of internal organs of the two rabbits, but in the central nervous system there were some diffuse round cell infiltrations or granulomata. R 657 showed changes in the eyes in the form of retinal loosening, and disintegration of the lens and a retinal abscess with a large fungus colony (Pl. XV).

In the case of all the rabbits forming series 3, which had been alive three months after the first inoculation, *tuberculin tests* were carried out. This was done according to Mantoux in one of the rabbits' ear-lobes and with a quantity of tuberculin up to 1 mg. *Negative results* were obtained in all cases.

Diagram 9.



Tests have been carried out in order to ascertain whether exotoxins had assisted in the pronounced diminution of weight which occurred in the majority of the rabbits inoculated with glycerin bouillon cultures of the fungus in question. 10 rabbits were inoculated in this way, 2 for each fungus strain, with 10—30 days old glycerin bouillon cultures after passing through a Zeiss bacteria-proof filter, and by testing the sterility of the filtrate. The animals were inoculated five times, twice with 1.5 cc and three times with 2 cc, but *without loss of weight* or other morbid symptoms occurring.

## CHAPTER VII.

### Summary of Morbid Symptoms, Pathological Changes, and Bacteriological Findings in the Rabbits Inoculated with the Fungus in Question.

Within 24 hours after intravenous inoculation with cultures of the fungus strains there became manifest in the rabbits a rise in

temperature from normally  $37^{\circ}\text{C}$ — $37.6^{\circ}\text{C}$  up to a maximum of  $40.1^{\circ}\text{C}$ , which after 2—3 days was succeeded by hypothermy with a minimum of  $34.8^{\circ}\text{C}$ . These temperatures were measured in a series of full-grown, large animals, none of which died of its infection. Two rabbits inoculated with the same quantity of commercial yeast manifested analogous, though small changes of temperature.

Often there occurred a decrease in weight in the rabbits after inoculation with fungus culture<sup>1</sup>, which with repeated inoculations was striking, or the weight of the animals kept fairly constant instead of rising in a manner natural for growing animals. During a longer or shorter period an increase in weight might remain absent or a low weight persist before the animal died *cachectic*, which was often the case, R 518, 519, 520, 521, 540, 555, 557, 559, 572, 586, 587, 590, 596, 598, 652, or the weight rose to normal values, R 541, 553, 560, 566, 595, 597. In tests for the purpose of ascertaining whether the decrease in weight was caused by the live fungus cells or their possible endo- and ectotoxins, the two last-mentioned ones in the doses used (see p. 35 and 49) did not show any influence upon the weight of the rabbits.

A certain amount of general atrophy ensued also with the diminution of weight, most easily noticeable in the spleen, which in the cachectic animals might be very small. In these animals there existed also a brittleness of the skeleton, so that in five rabbits an extremity was fractured when the animals were being stretched on the section board, though the utmost care was exercised not to use any force.

During their lives many of the rabbits inoculated with fungus culture manifested signs of infections of the eyes and nervous system, and these symptoms are stated in connexion with the following synopsis of the organic changes. Of these were found partly such as had with certainty been caused by the fungus cells, and partly such as had been due to infections contracted in the stable.

One rabbit, R 591, which died with distinct signs of weakness of the heart, showed in the right chamber verrucose, mycotic endocarditis, where the verrucose vegetations mostly consisted of large

<sup>1</sup> In the said synopsis of morbid symptoms and of changes of the organs no animals with certain stable infection (coccidiosis, pasteurellosis, spontaneous encephalitis caused by "*Encephalitozoon cuniculi*") are included, unless this is expressly stated.

masses of fungus cells (Pl. IX, fig. 1). In another case, R 589, were seen in the myocardium some solitary, granulomatous foci of epithelioid cells, but without any central necrosis. In the surroundings necrotic, calcinated muscle filaments. Twice there was encountered slight, respectively pronounced, myocardial fibrosis, R 588, 656 (the former died of coccidiosis hepatis).

In many cases no changes of the *lungs* resulted, R 540, 541, 553, 555, 557, 560 and several others, or only minor ones in the form of atelectases, haemorrhages, oedema and slight accumulations of round or plasmatic cells, e. g. R 521, 565, 566, 567, 586, 652, 653. Twice there occurred granulomatous foci of epithelioid and round cells, but without necrosis, R 564, 570, of which in the former fungus cells were seen in smears, while in the latter, which showed giant cells in the granulomatous foci, no fungus elements were encountered. In one case, R 527, there occurred granulomata of epithelioid and round cells mixed with uncharacteristic giant cells, as well as focal, caseous necroses containing degenerated fungus cells, acid-fast rods (Pl. VIII, fig. 1) and gram-negative, bipolar rods (see account of this p. 39). Twice there occurred abscesses with fungus cells, R 591 and 593. In the latter animal, which also showed bilateral sero-fibrinous pleuritis, were observed in the surroundings of some of the abscesses uncharacteristic giant cells and in larger or smaller arterial branches purulent thrombotic masses (septic emboli). On cultivation from the lung there was only obtained a growth of short, bipolar, gram-negative rods. In one case, R 518, suspected fungus cells and remnants of such were found in a necrotic focus with central colliquation. Many times there predominated in the lung-picture bronchitis, peribronchitis, and bronchopneumonia, largely certainly caused praeagonally (microscopically fresh changes). In smears from the lungs of two rabbits with bronchopneumonia, R 554 and 561, were found fungus cells.

In the *liver* of some of the rabbits occurred minor round cell infiltrations, e. g. R 520, or submiliary to miliary granulomata of epithelioid and round cells, and in a couple of cases with giant cell formation but without necrosis in the granulomata, R 527, 564, 566, 568, 570, 656, 657. Macroscopically these changes as a rule manifested themselves as small, slightly raised, whitish nodules. In the hepatic granulomata from one of the above-mentioned rabbits, R 527, were found plenty of acid-fast rods, and in another, R 564, solitary

fungus cells. Some of the animals, R 521, 561, 587, 590, 591, 598, 652 (Pl. IX, fig. 2 and Pl. X, fig. 1) showed diffuse necroses in the liver, and in smears from four of them, R 561, 591, 594, 598, were found fungus cells. In the necroses of R 587 no fungus elements were seen, but on the other hand in one of the major bile-ducts necrotic exudations stained and unstained, round and ovoid fungus cells (Pl. X, fig. 2).

The *spleen* was in many cases, particularly in the cachectic animals, strikingly small, and it was possible sometimes to observe microscopically fine atrophy of the parenchyma with close-lying trabeculae. Often there was present a more or less pronounced haemosiderosis in the reticular cells of the pulp, occasionally with giant cell formation. But in two out of four old, non-inoculated rabbits where the spleen was examined microscopically, there was found pronounced haemosiderosis in the pulp, and in one of them, partly in the process of giant cell formation. In the spleen of one of the animals, R 518, inoculated with fungus culture, there occurred miliary epithelioid cellular granulomata, but without giant cells and necrosis, and in another, R 527, quite small granulomatous foci, partly with polynuclear giant cells, but here, too, without necrosis. The foci contained a fairly large quantity of acid-fast rods (Pl. VIII, figs. 2 and 3), which on being cultivated on Hohn-medium grew in pure culture (Pl. XXII, fig. 5): (As regards tests with these rods, see Chapter IX.) In no instance were certain fungus cells met with in the spleen.

In those rabbits that died within the next few days after intravenous inoculation, the *kidneys* seemed often to be strewn with a large quantity of whitish nodules the size of a pin's head and smaller, which contained fungus cells. If the animals died after about 2—3 weeks, the nodules seemed to be still more fibrotic, so that the animals after about 1 month as a rule showed only more or less pronounced cicatrose pits on the surface. An example of the microscopic picture of a kidney from a rabbit that had died one week after inoculation with fungus, is R 589: Both in the marrow and cortex were seen numerous submiliary abscesses surrounded by more or less distinct granulomatous zones without necrosis. In this granulomatous tissue there occurred in several places rudimentary giant cell formations, possibly representing epithelioid proliferations from duct remnants. In smears from the kidney abscesses were seen quite a good many fungus cells and also some solitary

short, acid-fast rods, and in cultivation on Hohn-medium was obtained growth of both fungus and short, acid-fast rods (Pl. VI, fig. 2). — Two further findings of such rods were made in kidney abscesses (in animals that are not included in the rabbits mentioned in Chapters V and VI), once in a rabbit that died 5 days after inoculation with fungus culture, and the second time in a rabbit which had been begun to be inoculated with a mixture of cultures of the five fungus strains (Pl. VI, fig. 3) for the production of immune serum.<sup>1</sup> — In exceptional cases abscesses containing fungus cells could exist quite a long time, 3 months subsequently to the conclusion of the inoculations, R 652 (Pl. XI, fig. 1). In smears from this were seen plenty of fungus cells, some odd ones of which were acid-fast with Hallberg-stain, but the majority not stainable, although the counter-staining was done for a longer period than usual. Even with Gram-staining the fungus cells did not take any stain at all, or only poorly (Pl. VII, figs. 1 and 2). In the kidneys there existed in several cases diffuse, solitary round cell infiltrations and granulomata, e. g. R 520, 541, generally located radially through the cortex and often also the marrow, passing through more or less thick striae of connective tissue with generally gradually decreasing numbers of minor granulomata or round cell infiltrations, R 560, 586, and others (Pl. XII, fig. 1).

No visible changes ever occurred in the *digestive tract* (only a couple of cases have been examined microscopically). In the few animals in which the *suprarenals* were examined these showed, apart from submiliary granulomata and a couple of thin necrotic striae, nothing pathological.

In a large number of cases, 26 out of 42 rabbits inoculated with glycerin bouillon culture (furthermore also in a few inoculated with agar culture), there occurred disturbance of balance or paresis (Pl. XIII). Not once did there occur any signs of injuries in the nervous system in direct connexion with an inoculation. At the earliest, the symptoms in question made their appearance after 3, 6, and 7 days, in respectively R 561, 564 and 597. In the remaining cases the corresponding periods were from 2 weeks to 3 months,

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<sup>1</sup> Furthermore, out of 15 rabbits inoculated in the testicles with glycerin bouillon cultures of the fungus strains two, which died after 3 and 5 weeks, respectively, showed local abscesses containing fungus cells and short acid-fast rods. Moreover, in one of the animals was observed a large suspended abscess as well as caseous transformation of the corresponding glands.



in exceptional cases longer, but most commonly 2—6 weeks after the last inoculation. The disturbances consisted in difficulty to maintain the balance, awry head, pareses of varying degree to paralysis in the hind legs, but also some time in the trunk. It was only in exceptional cases that feebleness was manifest in the forelegs or in the musculature of the neck.

Amongst the *brain* and *spinal cord* from 40 animals (35 of these suffering from paralysis or paresis) that had been microscopically examined, mostly both were examined, fungus cells were found in only 3 cases, R 561, 596, 598. But it should be emphasized that no serial sectioning of the central nervous system was carried out, but only 3—6 sections had been taken from the cerebrum, generally just as many from the cerebellum, and 5—10 sections from the medulla oblongata and the medulla spinalis. R 561 displayed both in the cerebrum and in the cerebellum, especially in the cortex cerebri, multiple miliary and minor abscesses with a slight tendency to necrosis, and also in the surroundings considerable astroglial reaction and perivascular round cell infiltrations (Pl. XII, fig. 2). In some of the abscesses were found fungus cells in typical agglomerations (Pl. VII, fig. 4). In R 598 was observed in one cornu ammonis an abscess the size of a walnut with large quantities of faintly stained, or quite unstained, fungus cells. Moreover, there were seen in the stamgangliae hyperaemia and perivascular round cell infiltrations, as well as in solitary spots small glious foci. The same changes occurred also in one cornu ammonis. In a section from the brain of R 596 was found in the fourth ventricle a lump of exudation hardly the size of a pin's head, the lumen of it being occupied by a fungus colony. In the surroundings was seen a trifling ependymic proliferation. Changes were noticed also here and there in the cerebrum, partial destruction of the pyramidal cell band with glious proliferation, and, in some diffuse spots in the hemispherical marrow, perivascular round cell infiltrations. In 6 cases were found stable-encephalites with well-known etiology, R 693, 552, 560, 569, 585, 600. The changes in the brain consisted here of submiliary to miliary granulomata with central caseous necrosis, which contained plenty of corpuscles that were acid-fast with Hallberg-staining, and were of the same size and outline as those which Levaditi has named *Encephalitozoon cuniculi*. In these animals there occurred also diffuse granulomata without necrosis and round cell infiltrations. In

all the brains examined occurred, with few exceptions, changes in the form of locally diffused, submiliary, mostly perivascular round cell infiltrations, sometimes also in the meninges, and granulomata as well as minor gliotic foci. Moreover, occasionally were seen degenerative changes in the central ganglia and pyramidal tracts, as well as diffuse malacic foci. Only once were there found macroscopic changes, viz. severe, unilateral hydrocephalus internus. In the two rabbits which had been inoculated with ordinary commercial yeast, were seen perivascular granulomata. Round cell infiltrations, granulomata, and gliotic changes were met with also in two out of four brains examined from non-inoculated rabbits, which for over one year had been kept in the stables together with the inoculated ones. Out of the approximately 100 animals which had not been inoculated with live fungus culture, and which I have had an opportunity to observe for nearly two years, there occurred only one case of paresis in the hind legs (one out of a series of 50 rabbits which had been inoculated with dead fungus culture and then inoculated with tubercle bacilli). The central nervous system displayed in this case only uncharacteristic, perivascular round cell infiltrations.

Of the rabbits inoculated with the five fungus strains, macroscopic infections of the *eyes* were found in 12, viz. R 554, 558, 560, 566, 568, 570, 572, 586, 595, 600, 652, 657. In these animals there appeared at the earliest 5 weeks and latest 6 months, in the majority between 2 to 3 months, after the last inoculation unilateral or generally bilateral hyalitis oculi. On making a direct inspection of the eye it was possible to see through the wide pupil, at varying depths in the vitreous body, a light-reflecting tissue consisting of large, reddish-yellow to yellow, rounded formations (more or less vascular protrusion of the retina and hyalitic abscesses?), (Pl. XIV, fig. 1). Some time the vitreous body might appear diffusely greyish with somewhat floccose contents. Simultaneously with these changes there occurred sometimes more or less pronounced hyperaemia in the iris. At the earliest 3 weeks, generally a month or so after the appearance of the changes in the vitreous body, was noticed commencing clouding of the lens (Pl. XIV, figs. 2 and 3). A striking thing was that the animals quite a long time after the beginning of the ocular symptoms, retained some of their sight.

Microscopically there were present pronounced endophthalmitic

changes (such were met with also in one rabbit, R 563, which in life had not shown any distinct infection of the eyes): There were seen retinal and subretinal exudations; subretinal or retinal, often necrotising, abscesses; partial or total loosening of the retina, and proliferation of its glious elements; as well as granulomata, some of which contained plenty of lipid cells. In the vitreum occurred an increased content of albumin, diffuse agglomerations of cells and large abscesses. In the lens was sometimes seen periferal disintegration or necrosis within the capsule, occasionally with calcination. The chorioidea displayed granulomata, and, sometimes calcinations, and in one case, R 558, composite foci of submiliary round and epithelioid cells, which reminded one of tubercles or changes in sympathetic ophthalmia. Furthermore, there occurred in some eyes also signs of chronic iridocyclitis. In 7 of the rabbits were encountered in one or more abscesses fungus cells, sometimes solitary ones, sometimes in large colonies of round and ovoid cells mostly in the centre and in the periphery elongated with claviform swellings. Some of these colonies were surrounded by fairly broad, hyaline borders (Pl. XV, figs. 1 and 2). In the large colonies only the outside outlines of the fungus cells were noticed to have taken the stain like a nucleiform formation which in the elongated cells continued as a spirally twisted filament. In two of the rabbits, R 568, 652, the former with stable-encephalitis, there were found in abscesses a couple of small groups of fairly short, slightly clumsy acid-fast rods (Pl. VII, fig. 3), in the former case together with solitary fungus cells, and in the latter without fungus elements.

From the subjoined table of the rabbits<sup>1</sup> inoculated with glycerin bouillon culture of the fungus in question is seen partly with which fungus strain they have been inoculated, and partly the number of animals from which culture tests have been carried out, and in which case growth of fungus has been obtained, and partly the number of animals in which fungus elements have been found in the internal organs by culture or in direct preparations, as well as animals in which it has been possible to demonstrate fungus cells in one eye or both, as well as in the central nervous system.

Out of the 42 rabbits in the table, 10 were inoculated with strain II, and with the other strains 8 animals each. Of the 7 animals

<sup>1</sup> The numbers of the rabbits are in brevier, and the Roman figures state the fungus strain inoculated.

Length of life of rabbits after last inoculation	< 10 days	10—30 days	1—2 months	2—3 months	3—6 months	> 6 months
Number of rabbits inoculated with glycerin bouillon cultures	7 R 561II, 563II, 562II, 580II, 571V, 580II, 593II, 594II	3 R 599IV, 654V, 655V	3 R 565III, 567IV, 591II	7 R 529IV, 564III, 569IV, 597I, 590II, 598III, 653IV	8 R 526I, 527II, 528III, 530V, 559I, 572V, 592II, 652IV	14 R 558I, 560I, 566III, 568IV, 570V, 585I, 586I, 588I, 585II, 596III, 597III, 600IV, 656V, 657V
Number of rabbits from which culture tests have been made	5 R 561, 562, 571, 580, 593	1 R 599	2 R 567, 591	2 R 598, 653	3 R 527, 530, 592	2 R 558, 586
Number of rabbits from which fungus growth has been obtained	3 R 561, 562, 589		1 R 591			
Number of rabbits from whose internal organs in preparations and by culture fungus elements have been found	7 R 561, 562, 563, 571, 589, 593, 594	1 R 599	1 R 591	4 R 529, 564, 587, 598	2 R 527, 592	
Number of rabbits in which fungus elements have been found in one or both eyes					2 R 572, 652	7 R 558, 586, 595, 596, 597, 600, 657
Number of rabbits in which fungus elements (not Entcephalitozoon cuniculi) where found in the central nervous system	1 R 561			1 R 598		1 R 596

that died within 10 days, fungus cells were found in some internal organs of all of them, 6 of them being inoculated with strain II. In culture from organs in five of these cases growth of the fungus was obtained only in 3, and in the other cultivations made, only in one. After a fairly long time had elapsed between inoculation and autopsy, fungus cells were found in the internal organs more and more sparsely, and after 6 months none at all. A long time after the inoculation there were, however, found fungus cells in one or both eyes of 9 rabbits, in 7 of them 6 months afterwards. In the central nervous system fungus cells were found only 3 times.

In the rabbits inoculated with glycerin bouillon culture were also found, though this is not seen from the table, acid-fast rods in kidney abscesses of one, R 589, and in eye-abscesses twice, R 568 and 652, and in lungs, liver, and spleen once, R 527. Growth of both fungus elements and acid-fast rods was obtained from organs of R 589 and 591, but these findings will be dealt with in Chapter VIII. Growth of only acid-fast rods was obtained from liver and spleen of R 527. An account of this will be rendered in Chapter IX.

## CHAPTER VIII.

### **Inoculation of Cultures Containing Fungus Cells as well as Acid-fast Rods into Guinea-pigs and Rabbits.**

As described in the second part of chapter IV, acid-fast rods appeared in five glycerin bouillon cultures amongst the fungus cells, viz. two of strain I, one of strain II, and two of strain III. Altogether five guinea-pigs were inoculated with three of these cultures.

One guinea-pig, G-p 238, was inoculated subcutaneously in the right groin with 1.5 cc of the 35 days old fungus culture from strain I, in which only few acid-fast rods were found. A week later an abscess<sup>1</sup> fully the size of a pea was palpated at the spot of inocula-

<sup>1</sup> In subcutaneous inoculation of about 50 guinea-pigs with cultures merely containing fungus cells of the strains in question, an abscess the size of from a pea to a walnut is often obtained after 1—2 weeks at the inoculation spot. As a rule, it is resorbed within some little time, at the outside 2 months, or it may fistulate in exceptional cases. The abscess contains fungus cells which occur more and more sparsely the longer the abscess remains. Some time even short, acid-fast rods may be found.

tion, but this had vanished after about 2 weeks. 5 months after the inoculation the animal was killed, and nothing pathological was proved macroscopically. Three guinea-pigs, G-p 319—321, were inoculated subcutaneously in the right groin with respectively 1, 1.5, and 2 cc of the 62 days old culture of strain I, where a large accumulation of acid-fast, granular rods had been found (Pl. IV, fig. 2). Only the animal which was given the largest dose, showed at the inoculation spot after 1 week an abscess twice the size of a pea, which 3 weeks later had disappeared. The guinea-pigs were killed after respectively  $2\frac{1}{2}$ , 4, and 9 months, when no macroscopic changes were observed in the internal organs or at the inoculation spot. Unfortunately, no inoculations were made with that culture of strain II in which plenty of acid-fast rods had been found (Pl. III, figs. 4—11, Pl. IV, fig. 1). One guinea-pig, G-p 239, was subcutaneously inoculated in the right groin with 1 cc of the other culture of strain III in which rather plenty of acid-fast rods had been found (Pl. V, figs. 1 and 2). No abscess occurred at the spot of inoculation. The animal died after  $2\frac{1}{2}$  months. The autopsy showed, apart from a moderate loss of weight, nothing pathological.

### Inoculations of Cultures of the Strains from Animal Passages.

Two guinea-pigs, G-p 333, 334, and one rabbit, R 687, were inoculated with glycerin bouillon culture originating from renal abscesses from R 589 which had been inoculated with strain II (see Chapter V, diagram 6 as well as Chapter VII) and which contained both fungus elements and solitary acid-fast rods. G-p 333 was given 1 cc intraperitoneally and the other guinea-pig 1.5 cc subcutaneously in the right groin. They were killed after respectively 3 and 8 months and did not show any pathological changes at the autopsy. The rabbit, which had received 1 cc intravenously, displayed after  $1\frac{1}{2}$  month paralysis in the back part of the body and died within 2 months after the inoculation. At the autopsy pronounced *cachexy* was found (loss of weight 690 gr.), but otherwise nothing remarkable. Microscopically was noticed pronounced haemosiderosis in the spleen, and in the brain diffuse malacic and glial foci, while the other organs did not display any, or only trifling, changes. No findings of micro-organisms were made.

From the liver of R 591 inoculated, too, with strain II (see the above-mentioned Chapters), a glycerin bouillon culture had been made. This culture showed fungus after 24 hours at 37° C, and within 7 days also plenty of acid-fast rods. In smears taken after 7 to 14 days' growth were found amongst the fungus elements the acid-fast rods largely arranged in bundles, which in size and outline showed similiarity with ovoid fungus cells (Pl. V, figs. 4—7). — These figures agree fully with those of Reenstierna (103) from 1912. — From a non-acid-fast mycelial branch seems to issue a formation of acid-fast rods (Pl. V, fig. 8). Amongst the fungus cells there occurred acid-fast rods partly lying close together, forming balls (Pl. V, figs. 9 and 10), and partly in large irregular agglomerations (Pl. VI, fig. 1). Even acid-fast fungus elements were found in Ziehl-Neelsen stained preparations (Pl. V, figs. 11 and 12). In an agglomeration of non-acid-fast fungus cells of ordinary size was noticed a larger acid-fast ovoid cell, ostensibly consisting of closely packed, acid-fast elements. At one end of this formation there projected a rod-shaped "handle" (Pl. V, fig. 12).

Three guinea-pigs, G-p 329—331, and 4 rabbits, R 683—686, were inoculated with the said culture. G-p 329 was given subcutaneously in the right groin 1.5 cc of the 7 days old culture, and G-p 330 and 331 intraperitoneally respectively 1.5 and 1 cc of the 11 days old culture. After 1 month G-p 329 had a healed fistula in the right groin (the animals were not observed in the interval). G-p 331 was killed after about 3 months and the remaining ones after 8 months. None of the animals displayed any appreciable changes of internal organs. Of the rabbits, R 683, which was inoculated intravenously with 1.5 cc of the 7 days old culture, manifested after 4 days paresis of the hind legs and died one day later. In the lungs were noticed haemorrhagic foci, and in the kidneys a remarkably large quantity of white nodules the size of a pin's head, which contained a large quantity of fungus cells, as well as solitary, short, clumsy, acid-fast rods. On making a culture from the kidney abscesses on glycerin bouillon, only growth of fungus was obtained. A microscopic examination was made only of the central nervous system, which displayed diffuse abscesses (Pl. XII, fig. 3), containing numerous fungus cells, some of which, lying in agglomerations, were plentifully provided with granules (Pl. VII, fig. 5), which imparted to the cells a certain likeness to the "cones" descri-

bed by Hallberg (43). R 684, which was inoculated with 2 cc of the 9 days old culture, died after 7 days. The rabbit had then lost 760 gr in weight. The lungs and kidneys displayed abscesses (Pl. XI, fig. 2), which contained plenty of fungus cells, while no acid-fast rods could be found. On making a culture from the kidney abscesses on glycerin bouillon, a growth of fungus was obtained, and in a smear after 6 days at 37° C acid-fast, fairly short rods lying in the form of a tuft, could be seen. R 685 was inoculated with 0.5 cc and 0.75 cc of the glycerin bouillon culture originating from R 591, 11 respectively 21 days old. 6 weeks after the last inoculation, when the rabbit had lost 850 gr of weight, there occurred moderate hyalitis oculi bilateralis. 9 months after the last inoculation, when the animal was killed, it showed cataracta oculi bilateralis. The rabbit was then well nourished and did not manifest any appreciable changes of internal organs. In both eyes were noticed pronounced changes, and in one eye could be seen in an abscess plenty of fungus cells surrounded by a hyaline border. R 686 which was inoculated with 1 cc of the 11 days culture, died after 18 days. The rabbit was lean, loss of weight 1,540 gr. It showed focal, purulent nephritis with interstitial, strong fibrosis, and beneath the surface of the kidney cortex solitary, small abscesses. In the abscesses were discerned some highly suspected fungus cells, but on cultivating in glycerin bouillon and on Hohn-medium no growth was obtained. In the mesenterium were found a few small cysts, which contained parasites, certainly belonging to the intestinal worms.

## CHAPTER IX.

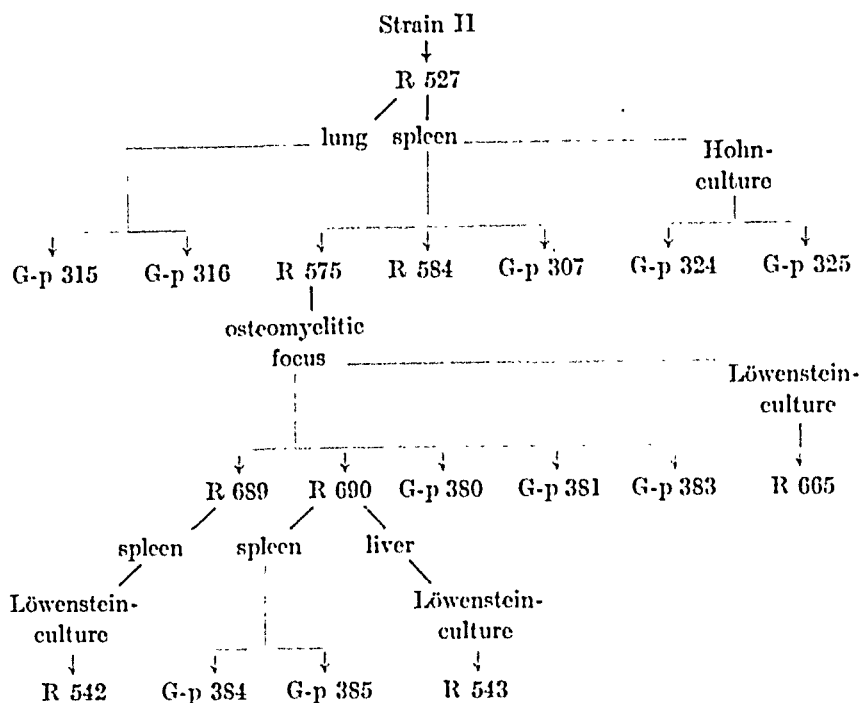
### **Passages in Rabbits and Guinea-pigs with Material (crushed organs and culture) from R 527, which had been Inoculated with Fungus Strain II.**

One of the rabbits inoculated with glycerin bouillon cultures from the fungus strains, R 527, as described in Chapter VI, manifested in the lungs, liver and spleen changes which reminded one of tuberculosis, and consisted in the lungs microscopically of granulomata of



histiozyten. Bei Besserung der Tuberkulose im Sinne einer produktiven Umwandlung kam es zunächst zu einer Vermehrung der Lymphohistiozyten, später, bei klinischer Heilung zu einer Normalisierung, mit Rückgang der Gesamtzellenzahl und Abnahme der Lymphohistiozyten. Da er die Lymphohistiozyten vom RES ableitet und die produktiven Veränderungen im Tuberkel ebenfalls durch Hyperplasie der retikuloendothelialen Zellen entstehen, führt er das Parallelgehen der granulomatösen Veränderungen in der Lunge und die lymphohistiozytäre Reaktion in der Kantharidenblase auf gleichartige Vorgänge im RES beider Organe zurück, wobei der Allergie eine grosse Rolle beigemessen wird. Kauffmann stützt sich dabei auf die Arbeiten von Rösle, der im Tierversuch auf den gleichen entzündungserregenden Reiz einmal überwiegend neutrophile, anderemale eosinophile oder lymphoide Zellen im akuten Entzündungsgebiet erscheinen sah, und das formale Geschehen des Entzündungsgebietes für eins der feinsten Symptome der eingetretenen Allergie hielt.

In diesem Zusammenhang müssen wir uns zunächst vergegenwärtigen, um was für eine Reaktion es sich bei der Kantharidenblase überhaupt handelt. Gänsslen hält den Kantharidenblaseninhalt für Gewebsflüssigkeit, deren Beschaffenheit von den im Gewebe zirkulierenden Kolloiden und Kristalloiden abhängt; er schränkt diese Auffassung zugleich aber ein und nimmt an, dass die Blasenflüssigkeit zum Teil auch direkt aus dem Blute stammt. Kauffmann wies in Schnittpräparaten nach, dass der Blaseninhalt Schlüsse auf die Reaktion des Coriums zulässt, so dass die Kantharidenblase unspezifische Reaktionen des Hautorgans wieder spiegelt. Wir haben es demnach mit einer, wenn auch nicht reinen, Organreaktion zu tun. Wir wissen aber schon aus anderen Gebieten der inneren Medizin, dass biopsische Untersuchungen von Organpunktaten eine Bereicherung unserer klinischen Methoden darstellen, wie das uns die Ergebnisse der Sternal-, Lymphdrüsen-, Milz- und Leberpunktion zeigen. Als eine leicht reproduzierbare Reaktion einer örtlichen Entzündung der Haut war die Kantharidenblase für uns von Interesse. Es sei schon hier betont, dass die Blasenflüssigkeit eine andere zelluläre Reaktion aufwies, als die Tuberkulinpapul, wie uns das systematische vergleichende Untersuchungen zeigten. Es sei ferner erwähnt, dass die Zellzusammensetzung in der Kantharidenblase nach unseren Befunden nicht vom Blutbild abhängt, eine Vermehrung der Lymphohistiozyten ging mit einer Blutlympho- oder Monozytose nicht parallel.



later weakness in the hind legs. When the rabbit died, 6 months after the inoculation, it was extremely *cachectic*. Outside the left temporal bone was noticed a caseous focus the size of an almond, which pushed the left eye forward, and microscopically proved to be a partly calcinated, caseous abscess, *very strongly reminiscent of an old, tuberculous, caseous focus*. It was surrounded by a fibrous capsule with *epithelioid cell granulomata with caseous necroses, greatly resembling tubercles* (Pl. XVII, fig. 2). On both elbows and knees, as well as in the left joint of the foot, were noticed large, knotty, caseous layers (Pl. XVI, figs. 3 and 4), and also near the thoracic part of columna vertebralis a caseous focus the size of a pea, all of them being found microscopically to be necrotic osteomyelitic foci surrounded by a border of epithelioid cells. In all the foci there occurred *acid-fast bacilli of Koch's type*, partly diffuse and partly in agglomerations (Pl. VIII, fig. 5). On the apices of the lungs were observed abscesses and otherwise small, whitish foci, which microscopically were found to consist of non-necrotic granulomata of epithelioid and round cells, and contained sparse *acid-fast rods*. The normal-sized spleen displayed macroscopically enormous haemosiderosis and diffuse *epithelioid*

*cell granulomata with numerous rudimentary giant cells, as well as a central necrosis with calcination and large quantities of acid-fast rods.* The kidneys manifested on the surface hyaline, cicatrose pits, and in the parenchyma some whitish nodules. Microscopically, there were seen cicatrose striae through the cortex, as well as *fresh cortical granulomata of masses of epithelioid cells and central caseous necroses with acid-fast bacilli.* Great similarity with tuberculosis. The ileum and caecum displayed on the surface plenty of yellow foci (Pl. XVI, fig. 2), consisting of granulomata lying in the submucosa with a moderate quantity of *acid-fast rods*. In the brain was found, apart from small, glious and insignificant perivascular granulomata, an older abscess with central, caseous necrosis containing *enormous quantities of acid-fast rods*, lying partly in bundles and partly in balls (Pl. VIII, fig. 4). R 584 was killed after 3 months, being then well-nourished. In the right lung was observed a largish, hyaline part which microscopically was seen to consist of an epithelioid cell-like proliferation of the alveolar walls. The spleen was of normal size, with a few white nodules the size of a pin's head (Pl. XVIII, fig. 1a), which microscopically was found to consist of *epithelioid cell granulomata with numerous rudimentary giant cell formations*, and one and another granuloma *commencing to turn into a caseous necrosis*. Rather plenty of *acid-fast rods*. G-p 307, which after 3 weeks at the spot of inoculation displayed a subcutaneous abscess the size of a hazel-nut, was killed after a further 3 weeks. No changes had taken place in the internal organs. In the right groin was noticed a subcutaneous abscess, which contained rather sparse *acid-fast rods*, and was surrounded by a membrane with the appearance of granulo-matous tissue rich in cells, with epithelioid cell granulomata in one spot, but without giant cells or necrosis.

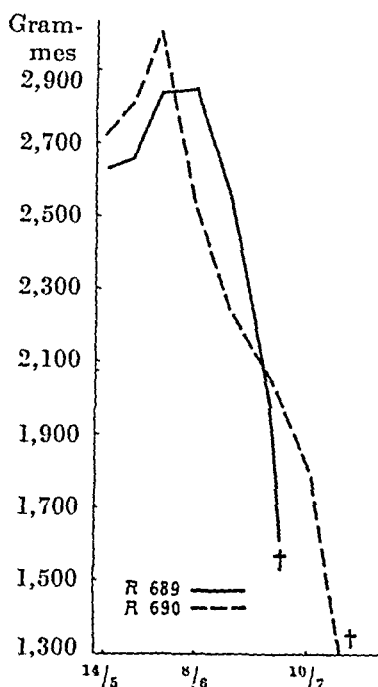
Two guinea-pigs, G-p 324 and 325, were inoculated subcutaneously in the right groin with 0.5 mg respectively 0.25 mg of the 57 days old Hohn-culture from the spleen of R 527. Both displayed after 10 days in the groin an abscess the size of a hazel-nut, which after quite 2 weeks fistulated. G-p 324 was killed after 9 months, and no pathological changes were found. G-p 325 was killed after 3 1/2 months. In the right groin were observed two abscesses the size of a pea, in places necrotic, and surrounded by a narrow border of epithelioid cells and containing plenty of *acid-fast rods*, separately and in agglomerations, some of them under phagocytosis (Pl. VII, figs. 6, 7). On

the right side of the arteria iliaca dexter was found a lymphatic gland the size of a hazel-nut kernel, which contained *epithelioid cell granulomata with central abscess formation*, but without necrosis.

From an osteomyelitic focus in the region of the left knee-joint of R 575 and the spleen of R 584, Löwenstein-cultures were made. In both growth of sulphur-yellow, moist, semiglobular colonies of *acid-fast rods* up to twice the size of a pin's head was obtained after 14 days (Pl. XXII, fig. 6). The third and following generations of the culture from R 575 showed, apart from the above-mentioned colonies, also larger, unevenly convex ones with pits in the centre (Pl. XXII, fig. 7).

A dab the size of a pea from the osteomyelitic focus in the left joint of the knee of R 575 was emulsioned in 10 cc NaCl-solution, after which two rabbits, R 689, 690, were inoculated with this intravenously with respectively 1 cc and 1.5 cc, and three guinea-pigs, G-p 380, 381, 383, the first-mentioned intraperitoneally with 1 cc and the other two subcutaneously in the right groin with respectively 1 cc and 1.5 cc. The two rabbits increased to start with in weight, after which there was a severe drop in weight. The animals died after respectively 1½ and 2 months. (See weight curve below.) R 689 showed severe *cachexy* and a *greatly increased spleen* (10 cm long) pervaded by nodules the size of a pin's head (Pl. XVIII, fig. 1), and microscopically pervaded by large and partly confluent *caseous necroses*, surrounded by thinner or broader *epithelioid cell borders*. Here and there *typical Langhans' giant cells*. Histologically, the changes were on the whole *identical with tuberculous changes* (Pl. XIX). The necroses were pervaded by *enormous quantities of acid-fast bacilli*, especially in the periphery, where they were lying partly diffuse and partly in large balls, and also partly in macrophagi and in the Langhans' giant cells (Pl. VIII, fig. 8). Nodules also occurred in the lungs, liver, and kidneys, though not so plentifully. Microscopically, the changes manifested themselves chiefly as *typical miliary tubercles*, having in the lungs moderate and in the liver plenty of *acid-fast bacilli*, diffuse and in bundles or balls. The nodules in the kidneys consisted of granulomata without caseous necrosis or giant cells, partly composed of epithelioid cells. Here and there *acid-fast rods*. R 690 showed at the autopsy severe *cachexy*. The lungs were pervaded by major or minor caseous foci (Pl. XVIII, fig. 2), and microscopically they displayed as a rule in the parenchyma, especially

Diagram 10.



below the pleural surface, *miliary and major tubercles*, with *caseous necroses* and purulent mixtures, and also in the surroundings diffuse *Langhans' giant cells* (Pl. XX). A moderate quantity of *acid-fast rods*. In the liver and normally sized spleen were observed plenty of nodules the size of a pin's head (Pl. XVIII, fig. 2), and microscopically *numerous miliary tubercles with caseous necroses and Langhans' giant cells* (Pl. XXI, fig. 1), and plenty of *acid-fast rods*, singly or in accumulations. Similar foci, though without typical giant cells, occurred also in the kidneys. G-p 380 died after 5 months and was then lean but manifested no pathological changes. G-p 381 lived in perfect health for another 7 months after the inoculation. In G-p 383 was after 14 days observed at the spot of injection a subcutaneous abscess the size of a hazel-nut, which was still there when the animal died about 5 months later. The guinea-pig was then lean. The abscess in the groin, which contained yellowish-white pus and fairly sparse *acid-fast rods*, was surrounded by a membrane with numerous epithelioid cells. A slightly enlarged lymph gland near the arteria iliaca displayed slight, chronic adenitis. No specific changes were observed in the internal organs.

0.2 mg of a 36 days old Löwenstein-culture (third generation)

from an osteomyelitic focus of R 575 was inoculated intravenously into a rabbit, R 665, which died after 3 weeks. Its lungs, liver, and enlarged spleen presented a fine-grained surface but no macroscopic foci. There were observed plenty of *epithelioid cell granulomata*, without necrosis. Only in the liver were found here and there rudimentary giant cell formations. Particularly in the spleen and liver occurred *remarkably plenty of acid-fast rods*, largely in bundles and balls. No specific changes were noticed in the kidneys.

From the spleen of R 689, from the liver of R 690, and from the inguinal abscess of G-p 383 Löwenstein-cultures were made, where the acid-fast rods grew after 14 days, and, in so far as the two first-mentioned were concerned, with colonies of the same appearance as described for the third generation of R 575 (Pl. XXII, fig. 8). The cultures from G-p 383 manifested, however, a different appearance. After a little more than 14 days the colonies were scarcely the size of a pin's head, greyish-white and not so moist (Pl. XXII, fig. 9). After another 2 weeks they were somewhat larger. Although the full-grown cultures were then kept at living-room temperature, the colonies assumed only a slightly yellowish tinge. Microscopically, it was impossible to distinguish the cultures. All of them contained only *acid-fast rods*, somewhat shorter than those observed in the organs in question.

0.2 mg of the 36 days old Löwenstein-culture from R 689 was inoculated intravenously into a rabbit, R 542. The latter died after 16 days. Apart from an enlarged spleen no macroscopic changes could be seen. The lungs manifested intimated bronchopneumonic infiltrations with a moderate amount of *acid-fast rods*. The liver was pervaded by granulomata with solitary minor giant cells, but no necrosis (Pl. XXI, fig. 2). Plenty of *acid-fast rods*, largely lying in bundles and balls. In the spleen, too, were observed granulomatous foci, indistinctly outlined, here and there with intimated necroses. The same findings of bacilli as in the liver. In one kidney was found a submiliary, granulomatous focus of epithelioid cells, surrounded by round cells. Some groups of *acid-fast rods* were encountered, inter alia in a couple of glomeruli.

A bit the size of a pea of the spleen from R 690 was crushed and emulsioned in an NaCl-solution, and inoculated subcutaneously into the right groin of two guinea-pigs each, G-p 384 and 385. In both there was manifested after about 2 weeks at the spot of injection an

abscess the size of a walnut and hazel-nut, respectively, which in the case of G-p 385 fistulated a week later. 3 months after the inoculation, when G-p 384 was being treated on its back with barium sulphide for the purpose of getting a surface free from hair for tuberculin tests, the guinea-pig died within 14 hours. The inguinal abscess contained fairly plentiful *acid-fast rods*. In a lymph gland near the arteria iliaca dexter were observed numerous submiliary epithelioid cell granulomata without caseous necroses, and without acid-fast rods. Only trifling microscopic changes were observed in the internal organs. G-p 385 lived in perfect health for another 5 months after the inoculation.

0.1 mg of the 36 days old Löwenstein-culture from the liver of R 690 was inoculated intravenously into a rabbit, R 543, which died after 5 weeks. The spleen was enlarged, while otherwise no appreciable macroscopic changes were manifest. Microscopically, there were observed in the lungs, liver and spleen *numerous miliary and submiliary tubercles*. Histologically, *great similarity with tuberculosis*. A moderate quantity of *acid-fast rods* was found in the said organs.

Tuberculin tests were carried out in five of the animals accounted for in this chapter, viz. R 665, 543 and G-P 380, 381, and 385. The rabbits were tested 16 days after the inoculation, G-p 380 and 381 after 5 months, and G-p 381 after 3 months. The tests with the rabbits were carried out intracutaneously in the shells of the ears, up to 1 mg of human tuberculin and 0.1 cc non-diluted aviary tuberculin being used, and on the guinea-pigs intracutaneously on the back, after the hair had been removed there with barium sulphide. In the tests with the guinea-pigs was used, apart from the said tuberculin, also a mixture of human and bovin tuberculin up to 1 mg. In no case was a typical positive tuberculin reaction obtained. On the sites for several of the tuberculin test-spots necroses were, however, obtained in the guinea-pigs two days after the injection, which was not seen in the controls with NaCl-solution. In order to be able to exclude the ability of the acid-fast rods to set up tuberculous allergy it will, however, be necessary to make more tests than have here been carried out.

Compared with R 527, the organic changes in the rabbits inoculated with acid-fast rods in culture, or the rabbits inoculated with organic material containing acid-fast rods, have *grown more and more resembling tuberculosis*, if we ignore R 665 and 542, which died respec-

tively 21 and 16 days after inoculation, which may possibly have been the reason why no caseous tubercles had time to develop. In all the changed organs were encountered *acid-fast rods of Koch's type*. They do, however, not appear to have been pathogenic for guinea-pigs, where with subcutaneous inoculation only an abscess occurred at the spot of inoculation, and besides in two cases changes in a corresponding lymph gland, in one of them with abscess formation with acid-fast rods.

## CHAPTER X.

### Discussion.

The initial material for the present investigation consisted of five fungus strains, isolated from sanatorium patients who were either being treated under the diagnosis of pulmonary tuberculosis, cases III and V, or had been under observation for suspected such, cases I, II, and IV. The five fungus strains agreed with each other in appearance, and with those that had been isolated by Reenstierna (103) from tuberculous material in 1912.

When the fungus had been cultivated over twelve months on ordinary agar tubes, single cell cultures were established of each strain by means of a micromanipulator, after which cultivation on ordinary slanted agar was made for 10 generations. It must therefore be considered out of the question that tubercle bacilli should after that have been present in the cultures when subsequent animal tests were carried out with the same, or with cultivations on media favourable for tubercle bacilli. Since we have not as yet any established method of classification for the fungi imperfecti, i. e. the yeast-like filamentous fungi, in which it has been impossible to demonstrate any sexual reproduction, I have not tried to classify my fungus-strains. The strains showed, irrespective of their varying capacity to produce acid-fast rods, varying but mutually the same cultural, tinctorial, and biochemical properties. On ordinary slanted agar the fungus grew in colonies which corresponded to the R- and S-form of the bacteria, of which the latter was the one most commonly occurring. Such dissociation has been described in yeast-



like fungi during the years 1937—1938 by Besta (12), Castellani (22), and Pinkerton (92). Two types of growth could be distinguished also in glycerin bouillon cultures, partly as cloudy, stringy masses, which might grow along the sides of the flask and out over the surface of the medium in the form of a soft pellicle, partly, though less commonly, as a granular growth only on the bottom of the flask. The stainability of the fungus elements varied to a high degree, according to the age of the culture, so that the acid-fastness with Hallberg-stain, and also gradually the general stainability, diminished with gradually increasing age of the culture. The ability of the fungus-strains to disintegrate certain carbohydrates during the development of gas, was investigated, when this ability proved capable of changing in the course of six months, something that Castellani showed possible to take place.

In five glycerin bouillon cultures from three of the fungus strains originating from single cells there appeared amongst the fungus cells acid-fast rods. In view of these findings one may ask how the acid-fast rods could be generated. No definite answer can be given to this question, but if all the observations made of acid-fast rods emanating from fungus cells are summarized, one can get a certain idea of the course of development. It is therefore necessary to compare my findings with previously made ones, viz. by Reenstierna and by Gullberg.

Reenstierna (103) observed in 1912, as mentioned in the historical survey, in two glycerin bouillon cultures made from single cells of a yeast-like fungus originating from tuberculous material, how the acid-fast rods, precisely like Koch's bacilli, which had appeared could lie ordered in formations that presented the same size and contour as the surrounding ovoid fungus cells. He considered that the acid-fast rods were formed in the fungus cells, which then disintegrated and evacuated their content. Some of his illustrations reproduce both ovoid, round, and elongated fungus cells in states of disintegration. That the acid-fast cells could develop in the manner stated, is apparent also from Gullberg's researches in 1933—1935 (41). An interesting formation, which probably constitutes the preliminary stage of such an ovoid formation as that described by Reenstierna, was observed by me in a glycerin bouillon culture originating from the liver of a rabbit, R 591, which had been inoculated intravenously five times with glycerin

bouillon culture of strain II (Pl. 4, fig. 12), viz. a large acid-fast cell, ostensibly consisting of closely packed acid-fast elements. In the upper part of this formation there projected a rod-shaped "handle". Furthermore, there were also found in the same culture acid-fast rods lying like ovoid formations (Pl. V, figs. 4—6), as well as conglobations of acid-fast rods (Pl. V, fig. 10), probably originated through propagation of the original rods lying in ovoid formations.

In two figures (Pl. IV, figs. 5 and 6) taken from Gullberg's work one imagines to see a course of development of the acid-fast rods slightly differing from the aforesaid. Fig. 5 illustrates between long fungus cells a non-acid-fast, amorphous mass with granules and very short rods, some of which are acid-fast. Fig. 6 shows a large, round formation, whose periphery consists of a wreath of ovoid fungus cells, while its centre is occupied by a net-work of blue filaments (Ziehl-Neelsen stain), in which there are acid-fast rods. These pictures seem to me to illustrate two stages of the formation of acid-fast rods.

Furthermore, I fancied several times that I had found in glycerin bouillon cultures of the fungus strains II and III some fine, unstained, mycelial filaments. Also stainable, shorter and longer, filiform elements have been seen. Thus Pl. III, fig. 5, illustrates rod-shaped ones, with acid-fast granules at the ends, while fig. 6 of the same plate shows filiform elements, of which some are acid-fast, whereas fig. 7 shows only acid-fast ones. Pl. IV, fig. 1, shows a ragged bundle of acid-fast rods and filiform elements, of which one of the latter seems to issue from a round fungus cell. The said filiform elements must have developed from the fungus cells, and the short, rod-shaped ones may have originated through fragmentation of the longer ones.

By way of summary one might be able to say that the acid-fast rods no doubt originate from an amorphous mass in the fungus cells, which via non-acid-fast granules and filaments differentiate into acid-fast elements. Sometimes, the outlines of the fungus cells seem to disappear first after this development, sometimes the fungus cells seem to disintegrate before its contents have been differentiated into acid-fast elements.

This must, of course, only be considered as hypotheses for a couple of probably several possibilities for the formation of the acid-fast rods, which is certainly of a very complicated nature.

Intravenous inoculation into rabbits of agar cultures of the fungus strains produced a change in the body temperature, analogous to that shown by Ostrowsky (87) with fungus isolated from thrush. In my animal tests were employed, as far as ever possible, not fully grown rabbits, which, with few exceptions, after the inoculations did not display the same rising weight-curve as the non-inoculated ones. Several of the animals died cachectic, something which had previously been pointed out by some authors, Reenstierna (103), Pijper (91), Gullberg (41), and Krauspe (59) after inoculation with yeast-like fungus. Often there was manifest distinct splenic atrophy, and the brittleness of the skeleton was striking.

The inflammatory changes of the internal organs were more pronounced, the sooner the animals died after inoculation, but not so generally disseminated as has mostly been described after inoculation with fungus of yeast type. This may be explained by lowered virulence in the fungus strains on account of prolonged cultivation on media — more than 12 months.

Inflammatory changes in the form of abscesses, round-cell infiltrations and granulomata were found mostly in the kidneys, and next to them in the liver, the lungs, the heart, and the suprarenals, and furthermore also degenerative changes, especially hepatic necroses. Only in one rabbit, R 527, were tuberculoid changes obtained in the lungs, liver, and spleen, which in the lungs consisted of granulomata of epithelioid cells mixed with giant cells up to the size of a pea, and also here and there in the granulomata caseous necroses, but in the liver and spleen of small granulomatous foci without necrosis. In the liver and spleen were met with acid-fast rods, which on being cultivated grew in pure culture, while similar ones in the lungs occurred together with gram-negative, non-acid-fast, pasteurilla-like rods. I shall revert later to attempts at explaining the origin of these foci.

In a large number of cases, 26 out of 42 inoculated with glycerin bouillon culture (furthermore also in a few inoculated with agar culture), there occurred disturbance of balance or paresis. Here the symptoms have possibly been obtained by co-operation between the fungus cells and other micro-organisms, inasmuch as the neurological symptoms only rarely occurred in the non-inoculated rabbits in the stable, while histological changes of the same nature, small granulomata, and in general perivascular infiltrations, could be

proved to exist in practically all rabbits, both inoculated and non-inoculated ones. In six cases there occurred furthermore changes in which *Encephalitozoon cuniculi* could be proved. Only in three cases were yeast-like fungi observed in the central nervous system.

In 12 rabbits occurred macroscopic ocular changes in the form of hyalitis, in the majority of cases with secondary cataract. Microscopically there was present considerable retinal destruction, with abscesses as well as necroses of the periphery of the lens.

In altogether 24 rabbits out of the 42 inoculated with glycerin bouillon culture of the fungus strains, fungus cells were encountered somewhere. Fungus elements were also found in some rabbits which had been given injections of agar culture. In 15 cases the fungus cells could be proved in the pectoral or abdominal organs, while a growth of the fungus in question was obtained by culture in only 4 cases. Of these animals, from which a growth of fungus was obtained, 3 had died within 10 days. Although the material is too scanty to enable safe conclusions to be drawn, the results nevertheless point to the possibility of finding fungus cells in the pectoral and abdominal organs, the same as the obtaining of growth by cultivation from here is greater a short time after inoculation than later. Of the 15 animals from which cultures were established, there were found in smears or sections fungus cells in 9, but cultures of these were only obtained in 4 cases. That a growth of fungus failed to take place, although fungus cells could be proved in smears, has been pointed out by Reenstierna, who considers this perhaps to be due to the fungus cells being in a state of disintegration.

Although the fungus cells could not be proved as a rule after about a month in the pectoral and abdominal organs, it was nevertheless possible, after a long time, to observe them elsewhere. After 6 months there was found a largish accumulation of fungus cells in the fourth ventricle of the brain of one rabbit, and after the same period in the eyes of 7 animals. A striking feature is that in so relatively many cases of ocular changes fungus cells could be proved after such a long time as six months. The explanation of this is probably that the content of antibodies in the vitreum in ocular infections, which Kuffler (61) has shown in 1912, is very small. He found with a content of antibodies in the blood of 1:10 000 that the same was 1:10 in the vitreum.

Acid-fast rods were observed in 4 animals out of those included in the series, in lungs, liver, and spleen of one, R 527 (whose organic changes have previously been mentioned in the discussion), in nephritic abscesses of one, R 589, and in ocular abscesses of two, R 568 and 652. Acid-fast rods were furthermore found in abscesses of another 4 rabbits, although not included in the animal series dealt with, but mentioned in Chapter VII. Although thus acid-fast rods could be proved in altogether 8 rabbits, organic changes caused by the acid-fast rods appear to have occurred only in one, R 527. The general condition of this animal, which suffered from an intercurrent affection, caused by gram-negative, non-acid-fast, *pasteurella*-like rods (see account of this in Chapter VI) was greatly impaired, which may explain the fact that the acid-fast rods were able to overcome the animal's power of resistance; and become pathogenic. There did not exist any basis for assuming that these were derived from the intercurrent affection, for which reason they must be considered as having originated from the fungus cells, as has been shown to be possible of appearance in cultures, and which seems to have occurred in the seven other rabbits where acid-fast rods had been obtained.

Altogether 9 guinea-pigs and 5 rabbits were inoculated with cultures containing both fungus cells and acid-fast rods, when no tuberculous changes were obtained. The circumstance that acid-fast rods, originating from fungus cells did not produce tuberculous changes, also Reenstierna found, and considered this to be due to the acid-fast rods only being a preliminary stage of tubercle bacilli.

With cultures containing merely acid-fast rods and derived from R 527, passages were made in rabbits, when tuberculoid changes were obtained, which in the second passage, R 689 and 690, were identical with those obtained after the inoculation of ordinary tubercle bacilli. Two rabbits, R 665 and 542, which died after respectively 3 weeks and 16 days, showed in the internal organs chiefly granulomata without necrosis, certainly due to the animals, dying so soon, that typical tuberculous changes had had no time to develop. In the first one of the inoculated rabbits no enlargement of the spleen existed, but in the continued and further passages considerable ones. In all the organic changes were as a rule found plenty of acid-fast rods of the same appearance as Koch's bacilli, lying partly spread about, partly in bundles, some of them being phagocyted,

and also partly in large balls. By subcutaneous inoculation of the acid-fast rods into guinea-pigs abscesses were obtained at the spot of inoculation, which in those animals that lived for a longer time, gradually disappeared. All the abscesses investigated contained acid-fast rods. Only in one guinea-pig was found a lymph gland with an abscess containing acid-fast rods, while the other organs did not manifest any specific changes.

Two rabbits and three guinea-pigs which had been inoculated with acid-fast rods, originating from R 527, were tested with up to 1 mg of a mixture of human and bovine tuberculin, without in any case typical tuberculin reaction being obtained. The same result was obtained in tuberculin tests of rabbits inoculated with the fungus strains.

On cultivating the acid-fast rods from R 527, these grew on Hohn-medium in 14 days in a temperature of  $37^{\circ}\text{C}$  in the form of moist colonies the size of twice a pin's head, while later generations showed unevenly convex colonies with pits in the centre. In culture tests at living-room temperature and temperatures of  $45^{\circ}\text{C}$ , no growth resulted. From a guinea-pig, G-p 383 — inoculated subcutaneously with material containing acid-fast rods from one of the rabbit-passages — cultivation was made from an abscess on the inoculation spot on Löwenstein-medium, when the acid-fast rods, after 14 days at a temperature of  $37^{\circ}\text{C}$ , grew to whitish, very slightly moist colonies of hardly the size of a pin's head, which after another 14 days were somewhat larger.

## Summary.

In a historical survey, Chapter I, is rendered a synopsis of the occurrence of yeast-like fungi in healthy human beings and in such with morbid changes, especially in the lungs, and furthermore of the possible relation of these fungi to acid-fast rods, as well as of the mycotic nature of the tubercle bacillus.

Chapter II covers a brief account of the histories of five sanatorium patients who were being treated for pulmonary tuberculosis or were under observation by reason of a suspicion of the same, and from each of which a fungus strain of yeast-type was isolated. In Chapter III is described the establishment of single cell cultures by means of a micromanipulator.

In Chapter IV is rendered an account of some morphological, tinctorial, and biochemical properties of the five fungus strains. Inter alia the capacity of the fungus to produce acid-fast rods is stressed there. Such rods have been found both spread out and lying in major and minor accumulations, and in certain peculiar formations that appeared in five glycerin bouillon cultures from three of the strains.

Chapters V and VI deal with animal tests, with intravenous inoculation of respectively agar and glycerin bouillon cultures of the fungus. The changes in weight of the test animals are illustrated in the majority of cases by diagrams. In Chapter VII is rendered a summary of the animal tests, symptoms and changes caused by inoculation being dealt with; important variations of temperature, often serious loss of weight, disturbances of balance and pareses, and also ocular and other organic changes. The changes of the internal organs were more pronounced shortly after inoculation than later on, with the exception of the eyes, where the changes became manifest late. In one rabbit, R 527, there occurred tuberculoid changes in the lungs, liver, and spleen, and from the two last-mentioned acid-fast rods of Koch's bacillus type were obtained in pure culture. Acid-fast rods were

tion, auch der Blasenbildung, bei Anergie, für die Tuberkulose nicht zutrifft:

Fall 7. H. M. 16j. Mädchen machte 8 Monate vor der Aufnahme eine tbc. Primärinfektion mit Erythema nodosum durch. Beim Eintritt war das volle Bild der generalisierten verkäsenden Tbc des ly. Systems mit grossen Drüsen feststellbar. Der Verlauf war unaufhaltsam progredient, Pat. starb unter den Erscheinungen einer therapierefraktären thrombopenischen Purpura und Peritonitis. Die Sternalpunktion ergab neben einer myeloischen Linksverschiebung eine aplastische Reaktion der Thrombopoiese. Sektion: Schwere verkäsende Tbc der thorakalen, und abdominalen Lymphknoten, der Milz, der Leber, ausgedehnte hämatogene Lungentbc. Die 10 Tage vor dem Tode angelegte Kantharidenblase wies morphologisch 70 % Neutrophile und 30 % Lymphohistiozyten auf, wobei Vakuolisierung und toxische Granulation der Neutrophilen (wie im Blutbild) und die Beteiligung der grossen, gelapptkernigen Zellen an der ly.-hist. Reaktion auffiel.

In diesem Falle bestand eine Tuberkulinanergie (Mantoux ausgewertet negativ) und auch der klinische Verlauf war anergisch. Die Kantharidenreaktion blieb aber nicht aus, im Gegenteil, wir erhielten eine ziemlich grosse Blase mit 0.9 ccm trübserösem Inhalt, der einen Eiweissgehalt von 6.7 % mit einem Albumin/Globulinverhältnis von 45/55 aufwies, während im Blutserum 7.85 % Eiweiss mit einem Albumin/Globulinverhältnis von 10/90 feststellbar war. Der Befund ist mit der grösseren Permeabilität der Gefässe für die feindispersen Albumine zu erklären, so dass es im Reizexsudat zur Anreicherung der Albumine kam. Das morphologische Bild mit 30 % Lymphohistiozyten sollte eher für eine günstige Prognose sprechen, der Krankheitsverlauf war aber unaufhaltsam progredient und der Tod trat 5½ Wochen nach dem Eintritt in die Heilstätte ein. Die relativ häufigen Versager schränken die diagnostisch-prognostische Bedeutung der KBR erheblich ein und sie ist auch für die Praxis technisch zu zeitraubend. Die Bedeutung der Methode scheint uns auf einem anderen Gebiet zu liegen; sie ermöglicht morphologische Einblicke in die Reagibilität des erweiterten RES, die uns bis jetzt in vivo noch versagt waren.

#### 4. Eiweisschemische Untersuchungen.

Die eiweisschemischen Untersuchungen sind für uns zunächst auch mit Rücksicht auf die Entstehung der Eiweisskörper im RES (Sabin, Heinlein, Rohr, Wuhrmann, Leitner) von Interesse.



changed appearance. The acid-fast rods could not be cultivated at living-room temperature or at a temperature of 45° C, nor on any medium unsuitable for tubercle bacilli.

In Chapter X the results of the investigation are discussed and theories for the origin of acid-fast rods from the fungus in question put forward.

## Appendix.

### Protocols of Inoculated Animals.

**R 691.**<sup>1</sup> Weight 2,795 gr. Killed 16 days after the inoculation.

*Autopsy:* Weight 2,725 gr. Small non-raised foci in the kidneys. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* The lungs show numerous perivascular infiltrations and endovascular changes in the small vessels. In some places granulomatous, small foci of large, light, plasmatic cells of round shape. The liver does not manifest any changes, and the spleen slight pulp haemosiderosis. In the kidneys are seen a couple of minor, moderately cicatrose striae with round cell infiltrations. The eyes without changes. The brain: In some odd places in the cortex perivascular round cell infiltrations and slight gliosis proliferation in the surroundings.

**R 692.** Weight 3,085 gr. Killed 16 days after the inoculation.

*Autopsy:* Weight 3,050 gr. No macroscopic changes of internal organs.

*Microscopic examination:* Lungs, liver, kidneys, and ventricle without any changes. In the spleen moderate haemosiderosis and partly in the course of giant cell development. The brain: In several places in the cortex perivascular round cell infiltration and perivascular granulomata with surrounding gliosis proliferation. The same condition exists in the central gangliae. In the wall of the third ventricle a compact round cell focus.

**R 693.** Weight 2,775 gr. Killed 16 days after the inoculation.

*Autopsy:* Weight 2,910 gr. No macroscopic changes of internal organs.

*Microscopic examination:* The liver does not show anything pathological, and the spleen trifling haemosiderosis. No changes in the eyes. The brain: In some few places in the cortex round, miliary necrotic granulomata, which contain dead-fast (with Hallberg stain), tiny bodies, which in size and shape agree with those that have been described by Wright and Craighead (133), and by Levallet, Nicolau, and Schen (68) in spontaneous rabbit encephalitis, and by them termed *Encephalitizon cuniculi*. Minor non-necrotising granulomata in some few places in the subcortical marrow. Perivascular infiltrations here and there in the central gangliae. Slight round cell infiltrations in the soft membranes around the cerebellum.

**R 694.** Weight 3,115 gr. Killed 16 days after the inoculation.

*Autopsy:* Weight 3,000 gr. No macroscopic changes of internal organs.

*Microscopic examination:* Lungs, liver, kidneys, and eyes do not show anything pathological, while the spleen displays moderate haemosiderosis. The brain: In some few places in the cortex miliary and submiliary granulomata, as well as perivascular round cell infiltrations, here and there also in the central gangliae.

**R 695.** Weight 2,575 gr. Killed 16 days after the inoculation.

*Autopsy:* Weight 2,590 gr. No macroscopic changes of internal organs.

*Microscopic examination:* The lungs and liver do not show anything pathological. In one of the kidneys is visible within the cortex and marrow an inflammatory focus

<sup>1</sup> The animals of which an account is given in this appendix occur in the same sequence as in the different chapters. Unless otherwise stated the animals were inoculated with any of the fungus strains, and the rabbits always intravenously.

the size of a hemp-seed, consisting of vast, interstitial round cell infiltrations. Atrophy of the duct system and chronic, productive glomerulitis, besides which numerous collecting ducts are dilated and filled with pus. The brain: Both in the cortex and the cornua ammonis solitary perivascular granulomata without necrosis.

**R 697.** Weight 3,000 gr. Inoculated with ordinary baker's yeast in maltose agar culture. Killed 16 days later.

*Autopsy:* Weight 3,160 gr. No macroscopic changes of internal organs.

*Microscopic examination:* No pathological findings in the liver, kidneys or eyes, while the spleen shows moderate haemosiderosis. The brain: In some places in the cortex small, perivascular granulomata with surrounding gliosis reaction.

**R 698.** Weight 3,240 gr. Inoculated like R 697. Killed 16 days after the inoculation.

*Autopsy:* Weight 3,300 gr. No macroscopic changes of internal organs.

*Microscopic examination:* Spleen, liver, kidneys, and eyes without pathological changes. Brain: In some solitary spots in the cortex of the cerebrum sparsely located perivascular granulomata.

**R 518.** Died 3 months after the last inoculation.

*Autopsy:* Pronounced cachexy. In the upper lobe of the left lung is seen a fibrosed focus the size of a large pea, with a fusion in the centre. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* In the lungs venous stasis with diffuse and focal interstitial bleedings with alveolar oedema. In one place, just below the pleural surface, a submiliary necrotic focus with central colliquation. The focus is surrounded by a concentrically stratified capsule of connective tissue and infiltrated with round cells, plasma cells, and leucocytes. In the firm part of the necrosis is seen a large number of round or oval bodies stained with haematoxylin, much larger than lymphocyte foci (suspected fungus cells), furthermore some acid-fast scalds and granules, probably remnants of fungus cells. The spleen shows strong atrophy of the pulp, with considerable coarse-grained haemosiderosis. Fibrous induration of the follicles, and in one or two places miliary epithelioid cellular granulomata without giant cells or necrosis. In the kidneys small fibrous scars, located radially in the cortex. In one of the scars an irregular caseous necrosis without fungus elements or remains of such, and without reaction in the surroundings.

**R 519.** Died 3 months after the last inoculation.

*Autopsy:* Cachexy. No macroscopic changes of internal organs. No microscopic examination.

**R 520.** Died 4 months after the last inoculation.

*Autopsy:* Cachexy. The spleen smaller than normal. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* In the lungs are seen minor areas with atelectasis as well as slight cell accumulations interstitially, and in the liver slight accumulations of round cells in the portal cords. The spleen shows remarkably strong pulp haemosiderosis, with coarse-grained pigment and parenchymatous atrophy, with closely lying trabeculae. In some few places in the kidneys are seen very small round cell infiltrations without necrosis. Suprarenals without remark.

**R 521.** Died about  $3\frac{1}{2}$  months after the last inoculation.

*Autopsy:* Cachexy. No macroscopic changes of internal organs.

*Microscopic examination:* The lungs present here and there slight atelectasis with oedema. In the liver are seen several necroses of submiliary size, clearly fresh, without cell reaction. No micro-organisms can be found. In the spleen moderate pulp haemosiderosis with slight induration of the follicles. The kidneys display some few very thin scars of connective tissue in the cortex, occasionally with trifling round cell infiltrations.

die Veränderungen nach Tuberkulin waren geringfügig. Immerhin nahm der Eiweissgehalt in 10 von 14 Fällen zu, in je 2 Fällen blieb er gleich bzw. er wurde niedriger. Das Albumin/Globulinverhältnis änderte sich bei 9 Kranken zugunsten der Albumine und bei 4 Kranken zugunsten der Globuline. Die Zunahme des Gesamteiweissgehaltes spricht dafür, dass es infolge der Allergisierung zu einer erhöhten Durchlässigkeit der Gefässe für Eiweisskörper gekommen ist. Die Zunahme der Albumine ist ebenfalls verständlich, weil die Gefässe zuerst für die feindispersen Eiweisskörper durchlässig werden. Die Globulinzunahme war bei 4 Schwerkranken feststellbar, bei 3 mit kavernöser Lungentbc und in einem Falle von doppelseitiger exsudativer Pleuritis. Es ist wahrscheinlich, dass bei Schwerkranken die Permeabilität der Gefässe soweit alteriert sein kann, dass sie dann auch die grobdispersen Globuline durchlassen. Zusammengefasst ergibt sich, dass nach Tuberkulinisierung eine Vermehrung der Gesamtzellzahl und der Neutrophilen, in geringerem Grade auch der Lymphohistiozyten eintrat, ferner eine Vermehrung der Gesamteiweissgehaltes, häufiger zugunsten der Albumine, seltener, namentlich bei Schwerkranken, auf Kosten der Globuline. Die Makrophagenreaktion, die sonst bei allergischen Zuständen beobachtet wurde (Kallós u. a.), war also in der Kantharidenblase nur geringfügig, doch unterscheidet sich letztere von reinen, akut allergischen Gewebsreaktionen.

Die Eiweissbefunde in der Kantharidenblase erklären vielleicht, weshalb in bestimmten Fällen eine exsudative Pleuritis zu fibrinreicher Exsudation mit starker Schwartenbildung führt. Bei schweren, entzündlichen Tuberkulosen kommt es bei einer bestimmten Allergielage zu einer vermehrten Permeabilität der Gefässe und zu Übertritt von grobdispersen Eiweisskörpern (Globuline, Fibrinogen) ins Exsudat (die Verhältnisse im Pleura- und Kantharidenblasenexsudat sind einander ähnlich). Praktisch noch wichtiger ist die Frage, ob es mittels der KBR möglich wäre, die Eigenschaft eines Exsudates im intra- und extrapleuralem Pneumothorax vor der Einleitung der Behandlung zu bestimmen? Es ist bekannt, dass eine stark fibrinöse »plastische« Exsudation zu vorzeitiger Verschwartung des Pnx führt, welche die Wirkung der Kollapstherapie vereitelt. Da die Beurteilung des Fibringehaltes der KBR einfach ist, hätten wir in ihr einen einfachen Test, die vor dem Eingriff (namentlich bei extrapleuralem Pnx) angestellt, wertvolle Hinweise geben könnte.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* Lungs, heart, and liver without any changes. The spleen displays moderate pulp haemosiderosis. In one kidney are seen in one spot in the margin of the marrow-cortex miliary granulomata, rich in cells, of uncharacteristic composition. The central nervous system: In the cornua ammonis and at places also in the cortex cerebri, as well as in the subcortical marrow, plenty of perivascular round cell infiltrations with surrounding glial proliferation.

**R 553.** 8 months after the last inoculation there appeared a permanent, moderate disturbance of balance. The animal was killed 3 1/2 months later.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* The lungs do not show anything noticeable, and in the spleen is seen a moderate pulp haemosiderosis. In the kidneys very numerous, fine streaks of connective tissue extending through the cortex down into the marrow, and in the liver in a couple of places submiliary granulomata in the parenchyma, with uncharacteristic giant cells. Very little similarity to tbc. Central nervous system: In one place in the cortex cerebri submiliary granulomata without necrosis.

**R 554.** 2 months after the last inoculation there was noticed hyalitis in both eyes. About a week later manifest loss of strength in the hind-legs, which later on showed distinct atrophy. 2 months after the appearance of the eye-symptoms was noticed a gathering of pus bilaterally in the pupil. The rabbit died 4 1/2 months after the last inoculation.

*Autopsy:* Emaciated. The forward parts of the lungs permeated by purulent foci. In smears are encountered some few solitary fungus cells, quite a good many "cones", as well as gram-negative rods. The abdominal organs without macroscopic changes.

*Microscopic examination:* The lungs display diffuse bronchopneumonia in the early stage of necrotic disintegration (gangraena?). In the liver there exists an uncommonly strong infiltration of round and plasma cells in the periportal connective tissue, but no sure parenchymal foci. The spleen displays hyperaemia and moderate pulp haemosiderosis, and the kidneys some solitary, minute scars in the cortex. Eye: Forward vitreous humour abscess and chronic iridocyclitis with some giant cell formations nearest the abscess. Furthermore, chorioiditis and retinitic changes. No certain fungus cells encountered. Central nervous system: Especially in the lateral parts of the cornua ammonis are seen perivascular round cell infiltrations. In the pedunculus cerebri, on one side, there is found a small infiltration focus of round cells and in one side of the lateral thalamus nucleus a necrosis the size of a poppy-seed, in whose periphery are lying peculiar formations, calcifications? Slight cell reaction in the surrounding areas. No certain fungus cells in the focus. In one place in the marrow of the vertebrae is seen a focus resembling that in the thalamus, though smaller. In its interior round cell infiltrations.

**R 555.** About 2 months after the last inoculation increasing paresis of the hind-legs. Died 2 weeks later.

*Autopsy:* Very emaciated. Contraction of flexors of hip-joints and atrophy in hind-legs. No macroscopic changes of internal organs.

*Microscopic examination:* Internal organs without changes. Central nervous system: Both in the cortex cerebri and some solitary central gangliae rather small, often perivascular, indistinctly delimited round cell accumulations. The same condition in the marrow of the cerebellum. In the spinal cord are seen not only perivascular round cell infiltrations but also round cell leptomeningitis and remnants of small granulomata, which had clearly been lying between the meninges.

**R 556.** 2 months after the last inoculation there appeared in the hind-legs moderately pronounced paresis, which was complete after 2 weeks. After another 3 weeks the animal showed dry gangraena in the left paw, which was then infected. Died quite 4 months after the last inoculation.

*Autopsy:* Cachexy. Infection in the left paw almost healed. The terminal phalanges of the paw protrude bare. Within the lower part of both lungs whitish, confluent

Untersuchung der Kantharidenblase im Vorversuch den Kranken 3 Tage lang täglich  $3 \times 15$  Tropfen Bellafolin (Sandoz) gegeben, nachdem sich uns dieses Präparat in früheren Ekg-Untersuchungen zur Dämpfung des Parasympathikus bewährt hat (Leitner und Steinlin). Da bei allergischen Reaktionen der Tonus des Parasympathikus erhöht ist, erschien uns diese Methode in Hinblick auf die Auffassung der KBR als allergische Reaktion (Kauffmann) aussichtsreich. Nach den 3 Bellafolintagen wurde erneut ein Pflaster aufgelegt und der Blaseninhalt nach 22 Stunden untersucht. Bei 7 derart untersuchten Kranken konnten aber keine verwertbaren Resultate erzielt werden, so dass eine Abhängigkeit der KBR vom veg. N. S. auf diesem Wege nicht nachgewiesen werden konnte.

### Summary.

A series of 221 patients was investigated morphologically and biochemically by means of the cantharides blister. Eosinophilia in the blisters was found in 36 patients, most of whom also showed blood and marrow eosinophilia. Parallelism between the number of eosinophils in the blood and in the cantharides blisters could not be established. Eosinophilia existed in the blisters even when absent in the blood, and on the other hand in one case eosinophilia was absent in the blister though marked in the blood. It must therefore be assumed that tissue eosinotaxis plays an important part in the production of eosinophilia in cantharides blisters. Of the 36 patients 28 also showed an increase of lymphohistiocytes. 68 patients showed neutrophil polymorph leucocytosis, and in 78 the lymphohistiocytes were increased. 43 of the patients with disease processes belonging to Ranke's secondary stage showed lymphohistiocytic and 19 neutrophilic reactions. Among the patients with tuberculous cavities we found 38 with neutrophilic and 20 with lymphohistiocytic reaction. When the disease took a favourable course the reaction of the cantharides blister was often characterised by an increase of lymphohistiocytes, but these changes were not sufficiently frequent. We must therefore disagree with Kauffmann and conclude that the cantharides blister reaction fails to give guidance for diagnosis and prognosis in tuberculous patients, even when used in serial examinations.

The time-consuming technique of the morphological examinations precludes the popularization of the method of the

mixed with uncharacteristic giant cells in large quantities. Here and there in the granulomata focal caseous necroses with remnants of disintegrated cell matter. The picture reminds one not so little of tuberculosis. In the liver are distinguished diffuse, very small granulomata, partly with polynuclear giant cells, but without any necrosis (Pl. XVI, fig. 1). In the spleen there occur quite small granulomatous foci of the same appearance as in the liver. Here and there small leucocyte foci; commencing abscess formation? No blood pigment. In the sections from the lungs, liver, and spleen a fairly large quantity of acid-fast rods is met with.

**R 528.** Weight 2,180 gr. After 4 months there occurred cataract in the left eye, and after a further 3 weeks moderate obliquity in the carriage of the head. The rabbit was killed 6 months after the first inoculation.

*Autopsy:* Weight 2,110 gr., lean. No macroscopic changes of internal organs. No microscopic examination.

**R 529.** Weight 2,515 gr. Died 9 weeks after the last inoculation.

*Autopsy:* The liver displays typical coccidiosis. No microscopic examination.

**R 530.** Weight 2,665 gr. Died slightly more than 3 months after the last inoculation.

*Autopsy:* Acute rhinitis. Weight 1,385 gr. No macroscopic changes of internal organs. No microscopic examination.

**R 558.** 6 weeks after the last inoculation there was manifested a weakness in the hind-legs. After a further 4 months there was noticed hyalitis in the left eye, and approximately 3 weeks later also in the right one. The rabbit was then, fully 6 months after the last inoculation, paralysed in the back part of the body. At the same time it contracted rhinitis and died after 4 days.

*Autopsy:* Brittleness of skeleton; the left fore-leg broke on the animal being stretched on the board for sectioning. On pressure being exerted on the lungs plenty of purulent matter exuded. Smears from the lungs contain small, gram-negative, bipolar rods, which on cultivation grow like pasteurella bacteria. The abdominal organs without macroscopic changes.

*Microscopic examination:* In the lungs purulent bronchitis without necrosis of the secretion. Pulmonary oedema and slight pneumonic infiltrations. Liver without change. The spleen displays pronounced haemosiderosis. In a section of kidney are seen some quite thin, cicatrose striae in the cortex, and solitary infiltrations. In the left eye there is present an old retinal loosening, in places with proliferation of glial elements and chronic chorioiditis, with solitary, submiliary foci resembling tubercles or changes in sympathetic ophthalmia. Furthermore, there is seen an abscess in corpus vitreum with necrosis and masses of fungus cells. The central nervous system: In some solitary places in the cortex submiliary granulomatous foci without necrosis.

**R 559.** 2 weeks after the last inoculation commencing paresis in the hind-legs. and a week later the rabbit sat with its hind-legs bent at the hip-joints but otherwise quite straight (Pl. XIII, fig. 1). Died 3 1/2 months after the last inoculation.

*Autopsy:* Position of hind-legs as stated above. Cachectic. Brittleness of skeleton. No macroscopic changes of internal organs.

*Microscopic examination* (only the brain examined): In the leptomeninx around the cornua ammonis slight round cell infiltrations. All sorts of degenerative changes in the nerve cells; postmortal changes?

**R 560.** 2 weeks after the last inoculation there occurred moderate paresis in the hind-legs and 7 weeks later hyalitis in the left eye. After another week was noticed obliquity in the position in which the head was held, and 6 months after the last inoculation hyalitis in the right eye. After another 6 weeks commencing cataract of the left eye. The rabbit had then difficulty in keeping its balance. It was killed 11 1/2 months after the last inoculation.

*Autopsy:* On the kidneys are seen minor scars. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* Lungs without changes. In some few spots in the liver are seen miliary and submiliary granulomatous foci, composed of epithelioid cells and minor forms of giant cells, and also surrounded by round and plasma cells. A certain similarity with tubercles is manifest, though there is no necrosis. The spleen displays severe, coarse-grain haemosiderosis, partly with giant cell formation. In a section of the kidney are noticed sparsely rather narrow and wider radially located cicatrose striae through the cortex, with trifling round cell infiltrations. In one place an interstitial round cell infiltration of miliary size. Both eyes display in the main analogous pictures: Close to the lens, which is partly infected at the edge, is seen a small hyalitic abscess. The central nervous system: In the cortex cerebri, subcortical marrow, and central gangliae small localised granulomata with surrounding glious proliferation. In one cornu ammonis a major granuloma, which fully agrees with the one described for R 603.

**R 561.** 3 days after the last injection there occurred a severe disturbance of balance. The rabbit was then dying, and was therefore killed.

*Autopsy:* The lungs show several white nodules, which in smears prove to contain fungus cells. On exerting pressure on the lungs there exudes a purulent secretion. On the liver are noticed some white, not raised foci, which in smears show fungus cells. The organs otherwise without visible changes.

*Microscopic examination:* The lungs manifest numerous bronchogeno abscesses and small purulent bronchopneumoniae. No very great tendency to caseous necrosis within the exudation, a suggestion of same within certain broncho-lumina. Moderate degree of haemosiderosis within the pulp of the spleen. In the liver are seen diffuse necroses. The kidneys manifest a form of chronic nephritis: Innumerable striae with plenty of cells within the cortex, where the glomeruli are atrophic. The straight channels are filled with desquamated epithelium. The central nervous system: In the front part of the cerebrum, far less than in the cerebellum, cortical foci in the form of abscesses with a slight tendency to necrosis. In the environs severe astroglia reaction and perivascular round cell infiltrations. In the cornua ammonis and pedunculus cerebri also in several places similar but smaller foci of decreasing intensity. In some of the foci are found fungus cells in minor units. In the cortex of the cerebellum, too, are seen a number of submiliary abscesses.

**R 562 and 563,** died 8 days after the last inoculation.

*Autopsy:* The kidneys are pervaded by abscesses the size of a pin's head. Otherwise no organic changes visible. In smears from the kidneys plenty of fungus cells. No sections of the organs examined.

**R 564.** 6 days after the last inoculation there became manifest a serious disturbance of balance, and one month later paresis in the hind-legs, which after a further 3 weeks were paralytic. Slightly more than 2 months after the second inoculation the animal was killed.

*Autopsy:* Emaciated. In stretching the animal on the board for sectioning the right femur is fractured. In the lungs and kidneys there are noticed small, white nodules, which in smears prove to contain solitary fungus cells. Otherwise the organs are without macroscopic changes.

*Microscopic examination:* In the lungs are seen major and minor granulomatous foci, whose centres consist of large, pale, plasmatic cells (epithelioid cells), and periferally round cell infiltrations. Nowhere caseous necrosis. Severe hyperaemia and atelectasis. The spleen shows pronounced pulp haemosiderosis. In the liver parenchyma, outside the portal vessels, are seen diffuse, small cell infiltrations and granulomatous foci, some of which latter contain polynuclear giant cells. In the kidneys are seen diffuse, scarified striae through the cortex in connection with round cell infiltrations, as well as here and there fresher granulomatous striae. The central nervous system: In the cortex cerebri and in the hemispherical marrow focal glious infiltrations. In the pedunculus cerebri are seen foci that rather resemble perivascular granulomata, though quite small. Slight pial round cell infiltrations, especially near the cerebellum. In



the basis of pedunculus cerebri none, or trifling, changes. In one of the spinal cord sections are seen in the pyramidal frontal tract slight perivascular round cell infiltrations.

**R 565.** Fully one month after the last inoculation there occurred the commencement of loss of strength in the hind-legs, and 2 months later the rabbit sat with its legs stiffly extended. After another 10 days that part of the back of the body which is most subject to pressure, presented a purulent, bloody mass, with the left testicle hanging down in its funicle, and therefore the animal was killed.

*Autopsy:* Right testicle missing, left one as stated above. Ulcerous surfaces in gluteal and perineal regions. No macroscopic changes of internal organs.

*Microscopic examination:* In the lungs are seen here and there slight parenchymatous blood effusions. The spleen is ostensibly anaemic, with only trifling pigment in the blood in the reticular cells of the pulp. The liver and kidneys show no changes. The central nervous system has not been examined.

**R 566.** 5 weeks after the last inoculation there was noticed in the hind-legs a loss of strength, which after a few weeks diminished. Approximately at the same time there commenced cataract bilaterally with gradually occurring vascular injection in the sclerae. After 2 months there was present endophthemia chronica oculi bilateralis. The rabbit was killed 11  $\frac{1}{2}$  months after the last inoculation.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* In the lungs there is visible only trifling atelectasis. The liver displays some solitary miliary foci composed of epithelioid cells and round cells without giant cells or necrotic formations. The spleen manifests severe, coarse-grained haemosiderosis, partly with giant cell formation. In the kidneys fine cicatrose striae through the cortex and a couple of major foci of connective tissue, with slight round cell infiltrations. Both eyes manifest mainly analogous conditions: Endophthemia chronica with encapsuled hyalitic abscess and glious retinal proliferations. Plenty of lipid cell accumulations around the abscess, which is seen to contain digested fragments of the lens, which is partly dissolved. The central nervous system: In the meninges, especially around the hippocampus and to a minor degree around the convexity of the brain, round cell infiltrations.

**R 567.** 5 weeks after the last inoculation there occurred progressive paresis in the hind-legs and the rabbit died after a fortnight.

*Autopsy:* Emaciated. No macroscopic changes of internal organs.

*Microscopic examination:* In a section from one of the lungs are seen minor atelectatic portions below the surface, but otherwise nothing remarkable. The liver is without pathological changes. In the kidney are seen generally small cicatrose striae through the cortex, some of them with round cell infiltrations. In one place a granulomatous focus, which is probably lying in the place for a destroyed glomerulus. The central nervous system has not been examined.

**R 568.** Slightly more than 1 month after the last inoculation there occurred moderate paresis in the hind-legs, which progressed during the next month, but then disappeared altogether. 4 months after the last injection there was seen hyalitis oculi dexter, and 7  $\frac{1}{2}$  months later, when the animal was killed, cataract in the same eye.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* The lungs present no changes. In the liver are seen at the border next to the portal connective tissue here and there submiliary granulomatous foci of the same appearance as in R 566. The spleen displays a good deal of blood infusions in the sinus and fairly strong haemosiderosis. In some reticular cells are seen grainy and different-sized pellets which are greyish in haemotoxylin-stained preparations and red in Hallberg-stained preparations, these often displaying stronger staining of the periphery. Their nature cannot be closely determined. In the kidneys very sparse narrow, radially-located striae of connective tissue, partly reaching down through the marrow, with trifling round cell infiltrations in a couple of places. One eye does not

present any changes. The other shows chronic iridocyclitis with old retinal loosening. In a Hallberg-stained section are encountered in a subretinal abscess two small groups of somewhat clumsy, acid-fast rods (in appearance agreeing with those in Pl. VII, fig. 3). The central nervous system: In the cortex cerebri, subcortical marrow, and, roughly outlined, in the cornua ammonis solitary granulomata analogous with those described for R 693.

**R 569.** 4 weeks after the last inoculation there appeared disturbance of balance and a week later moderate, but afterwards increasing, paresis in the hind-legs. Died 2 1/2 months after the last inoculation.

*Autopsy: Emaciated.* No macroscopic changes of internal organs.

*Microscopic examination* (only the spinal cord examined): No certain changes visible.

**R 570.** 2 weeks after the last inoculation there occurred moderate paresis in the hind-legs. 14 days later hyalitis oculi bilateralis was noticed. After another month there was present cataract in the right eye and after another 2 weeks also in the left one. The rabbit was killed 11 1/2 months after the last inoculation.

*Autopsy: Emaciated.* Apart from minor scars in the kidneys no macroscopic changes of internal organs.

*Microscopic examination:* In the lungs diffuse minor foci of large cells, partly epithelioid cell composition, reminiscent of small granulomata. No giant cells. No necrosis. The liver displays several diffuse, submiliary granulomata of epithelioid and round cells, as well as partly with giant cell formation. No necrosis. *A certain resemblance to tubercles.* In the spleen is distinguishable moderate haemosiderosis with giant cells. Sections from the kidneys show in the cortex a wedge-shaped, fibrotic focus with retained glomeruli. Trifling round cell infiltration. In one eye is present old retinal loosening of the edge of the lens, with lime incrustations. The other eye shows mainly the same picture, though with slight suppuration. The central nervous system: In the cortex cerebri, subcortical marrow, and cornua ammonis solitary, submiliary, probably perivascular, granulomata. In the spinal cord signs of fresh pyramidal tract degeneration.

**R 571.** Died 4 days after the second inoculation.

*Autopsy:* In the left pleural cavity 3 teaspoonful of exudation mixed with blood, but without micro-organisms in smears. The lungs without visible changes. On the left lobe of the liver some whitish, non-raised foci containing some fungus cells. Otherwise no macroscopic changes of internal organs. No microscopic examination.

**R 572.** 6 weeks after the last inoculation commencing, progressive paresis in the hind-legs. 14 days later hyalitis oculi bilateralis, and after a further 3 weeks cataract in the right eye. Died 4 months after the last inoculation.

*Autopsy: Cachectic.* No macroscopic changes of internal organs, apart from very minute scars on the kidneys.

*Microscopic examination:* In the lungs diffuse haemorrhages, otherwise nothing pathological. The spleen displays trifling blood pigment in the reticular cells of the pulp. Liver and kidneys without appreciable changes. Eye (only one eye examined): In the rear lateral part of the bulb a subretinally located abscess encased by the fibrosely altered, hardly recognisable, retina, whose neuroglia is in irregular proliferation. Inside the abscess is seen a fungus colony the size of a millet seed, mainly composed of round and ovoid cells. In the capsule of the abscess is also found a minor abscess in which sparse fungus cells are visible. The central nervous system: Perivascular, slight, diffuse infiltrations in the cornua ammonis and also in the meninges. Trifling round cell infiltrations in the meninges around the cerebellum. Otherwise nothing certain pathological.

**R 585.** Hardly 2 months after the last inoculation there occurred a slight disturbance of balance and some obliquity in the carriage of the head. The animal was killed 7 1/2 months after the last inoculation.

*Autopsy:* The spleen smaller than normal. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* Solitary submiliary epithelioid cell granulomata without necrosis in the lungs. Furthermore, purulent bronchitis with aspirated foreign bodies. Heart and liver without changes. In the cortex of the kidney are visible some solitary round cell infiltrations, and in the marrow a fibrose stria. The spleen displays trifling pulp haemosiderosis. The central nervous system: In the cortex cerebri and one side of the central gangliae slight granulomata. The picture is analogous to the one described for R 693. In the basis of the pedunculi cerebri and in the pons arms small, perivascular glial cell proliferations.

**R 586.** 4 weeks after the last inoculation moderate paresis in the abdominal muscles and hind-legs, and after a further 2 months hyalitis in the left eye, and a few days later also in the right one. 3 months later phthisis bulbi sin. Died  $7\frac{1}{2}$  months after the last inoculation.

*Autopsy:* Pronounced cachexy. General organic atrophy, especially the spleen remarkably small. In both kidneys plenty of scars. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* In the lungs severe hyperaemia and minor haemorrhages. The liver is without any changes. In the spleen exist parenchymatous collapse and considerable anaemia, as well as a trifling quantity of blood pigment in some reticular cells. In sections from the kidney the cortex is seen to be pervaded by a large number of fine cicatrose striae, with a small quantity of round cells, furthermore a large, strongly marked fibrous portion with retained glomeruli and plenty of interstitial round cell infiltrations. The area is delimited from the marrow by a broad, haemorrhagic, hyperaemic zone. In a papillary apex is seen a small focus of round cells and leucocytes. Testes without any changes. Left eye: Endophthemia chronica with an old abscess, which contains numerous fungus colonies. The right eye presents in principle the same picture, though with somewhat fewer masses of fungus elements. The lens is at the edge seen to be in a state of dissolution, and in the chorioida layer is seen a large necrotic granuloma. The central nervous system: One-sided, severe hydrocephalus internus. In solitary places in the cortex cerebri minor glial infiltrations, and in the subcortical marrow in several places perivascular infiltrations. In two places close to each other there are existing old malacic foci, one of which contains a small granuloma surrounded by proliferating glial cells. The granuloma cells contain in the centre masses of bluish-green grains (with Nissl-stain), and several necrotic interstratifications, partly of a granular-ropy structure. The second focus contains no granuloma, only some necrotic interstratifications. Very minute glious proliferations in the pons arms. The spinal cord without visible changes in the sections taken.

**R 587.** 18 days after the first inoculation obliquity of the head set in, and after a further 3 weeks increasing paralysis of the hind-legs, and then of the whole back part of the body. Died  $2\frac{1}{2}$  months after the last inoculation.

*Autopsy:* Pronounced cachexy. The back part of the body is atrophic. The left kidney shows several scars. On the back of the liver are seen some reddish-white, minor foci. The internal organs otherwise without macroscopic changes.

*Microscopic examination:* The liver presents some major and minor necroses with plenty of disintegration of nuclei. The foci are located close below the surface and appear to be perfectly fresh, without any surrounding granuloma zone (Pl. X, fig. 1). In several gall ducts are seen necrotic exudations, and in one of the larger ones are found plenty of fungus cells, which in Hallberg-stained sections are partly acid-fast, partly non-acid-fast, and partly not stained at all (Pl. X, fig. 2). In the kidneys the cortex seems to be permeated by numerous cicatrose striae, rather deficient in cells. The central nervous system: In the cerebrum, cerebellum, and pedunculus cerebri nothing certain pathological is noticed.

**R 588.** Died hardly 8 months after the last inoculation.

*Autopsy:* Cachexy. On exerting pressure on the lungs there exudes a light, purulent secretion. In the liver are seen several white foci, one of them being the size of nearly a pea. In the kidneys minor scars.

*Microscopic examination:* In the heart is visible, in foci, light myocardial fibrosis. The lungs display several coccidiosis foci without necrosis. In the liver there are several coccidiosis foci with calcifications. Furthermore, fibrose foci with haemosiderosis. In the spleen there exists strong pulp haemosiderosis, partly with giant cell formations. In the cortex of the kidney and down in the marrow fairly plentiful, fine radially located striae deficient in cells, and also minor fibrose foci. The central nervous system: In some odd places trifling perivascular round cell infiltrations, and in the cortex in one place a small focus composed of very minute granulomata and glial cell proliferations.

**R 589.** Died 7 days after the inoculation.

*Autopsy:* The kidneys permeated by white abscesses the size of a pin's head, and in the liver a few such. In smears from the kidney abscesses fairly plenty of fungus cells, as well as some solitary, short, *acid-fast rods*. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* The heart displays in some odd spots in the myocardium microscopic granulomatous foci, with diffuse epithelioid cells, without necrosis. In the surroundings necrotic, calcinated muscle filaments. In the lungs are seen some few bronchopneumonic foci of a purulent nature, as well as bronchites and atelectases. The liver manifests strong hyperaemia but otherwise nothing pathological. In the kidneys are seen in both marrow and cortex numerous submiliary abscesses surrounded by more or less distinct granuloma zones. In these granulomata in several places rudimentary, polynuclear formations, possibly representing epithelioid proliferation from duct remnants. No necrosis.

**R 590.** 5 weeks after the last inoculation increasing paresis of the back part of the body. Died hardly 3 months after the last inoculation.

*Autopsy:* *Pronounced cachexy.* Especially the spleen atrophic. Scars in the kidneys. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* The lungs do not present anything remarkable. In the liver, just near the border to a portal cord, a submiliary necrotic focus with numerous round cells, possibly belonging to the periportal tissue. The spleen displays collapse of the parenchyma and in several places blood pigment in the reticular cells, as well as plasma cells in the pulp. In a section from the intestine nothing pathological is noticed. The suprarenals show in the cortex in one place a slight, fresh necrotic stria. In the kidney diffuse, radiary, narrow striae through the cortex, here and there with sparse round cell infiltrations. The central nervous system: Light round cell infiltrations in the mucous membrane around the hippocampus, and in the region of the fissura sagittalis a preponderating round cell meningitic focus without necrosis. In the foot of pedunculus cerebri a small glious focus.

**R 591.** 1 month after the last inoculation the rabbit is seen to drag his bent hind-legs. Died 1 week later.

*Autopsy:* When stretching the animal on the board the left femur fractured just above the knee. Bilateral hydrothorax, in the belly a fairly large amount of a clear exudation, oedema on the back. The heart considerably dilated with coagulated blood in all chambers. In the right chamber are seen on the apices of the papillary muscles and a couple of the cordae tendineae white, verrucose excrescences the size of millet seeds and smaller, furthermore an apparently freely lying white, small nodular formation the size of a pea. The trachea considerably vascularly injected, and a moderately mucous coating. In the left lung's lower part a whitish focus nearly the size of a pea, and diffused over the lungs minor such. On the surface of the lung minor haemorrhages. The liver shows an uneven, slightly nodular surface with diffuse, small, whitish foci, which in smears contain a moderate quantities of fungus cells. *On cultivation in glycerin bouillon growth of fungus and after 7 days acid-fast rods.* The other internal organs without macroscopic changes.

*Microscopic examination:* Sections from the heart substance show solitary sub-endocardial layers. The excrescences on the papillary muscle apices the size of a millet seed, are composed microscopically of large, cohesive layers of fungus cells, with beau-

tifully radial configuration of the long cells, especially in the peripheries. A hint of concentric stratification. Between the fungus cells are lying masses of disintegrating embedded pus. The surface of the masses of fungus are in free contact with the blood. On the surface of a papillary muscle there is a miliary formation of abscess, surrounded by a thick, close capsule of connective tissue, which in its interior stratum contains some round cells and solitary uncharacteristic giant cells. In the centre of the abscess a small fungus colony with the same appearance as above and with numerous round and ovoid fungus cells. The free formation of the size of a pea is composed of radial fungus masses and detritus mixed with disintegrating pus (Pl. IX, fig. 1). In one place of the formation is seen a cross section of a corda tendinea, in whose necrotic periphery and surroundings are lying large masses of fungi.

Sections from the lung show in one place an older abscess the size of a pea, with necrosis in the centre. In the surroundings slight signs of subchronic pneumonia. The spleen shows very serious hyperaemia with maximally blood-filled sinus. Slightly pronounced haemosiderosis. In several places slight infiltrations of plasma cells. In the liver numerous, irregularly confined necroses without special location, and without manifest cell reaction in the surroundings. Within the necroses the star cells are surviving to a large extent. In some portal vessels fresh thromboses. Furthermore, plenty of blood pigment in the star cells. In the kidneys radiated and irregular connective tissue scars, chiefly in the cortex. Moderate round cell infiltrations in some of them. The central nervous system: In some few places in the cortex purulently infiltrated, round, submiliary necroses in whose surroundings are seen manifestly perivascular round cell infiltrations. The cerebellum and pedunculus cerebri show nothing certain. In the lumbar part of the spinal cord a glious focus permeated by round cells. In the surroundings hypertrophic astroglia.

**R 592.** 3 weeks after the last inoculation there became manifest a feebleness in the hind-legs, which 2 months later diminished. Died 4 months after the last inoculation.

*Autopsy:* On the back a scratch that is healing. The liver shows several whitish foci, which proves to contain pneumococci of type 19 in pure culture. Other organs without visible changes.

*Microscopic examination* (only the central nervous system examined): In the subcortical marrow some perivascular round cell infiltrations. Indicated granulomatous formation in the cortex in a couple of places.

**R 593.** Died 4 days after the last inoculation.

*Autopsy:* Sero-fibrinous pleuritis bilaterally. The lungs pervaded by white nodules the size of a pin's head, as well as larger foci resembling infarcts. The spleen without visible changes. In the liver and kidneys plenty of small, white nodules. In smears from the lungs and the kidneys sparse fungus cells, but on cultivation growth only of gram-negative rods.

*Microscopic examination:* The lungs pervaded by miliary and submiliary abscesses, which mostly appear to be fresh. In the surroundings of some of the abscesses uncharacteristic giant cells. In major and minor arteries of the lung purulent thrombotic masses. The picture agrees with septic emboli in the lung. The spleen shows strong post mortal autolysis, which does not enable a closer judgement. The liver is changed to a high degree in the form of major and minor, partly confluent, fresh necroses, which are mostly localised to centres of the acini. Furthermore, there appear diffuse, quite small, fresh abscesses. In several places thromboses in the finer portal vessels. The kidney displays in the cortex broader and thinner cicatrose striae with sparse round cell infiltrations. Furthermore, small fresh abscesses without any granuloma. In several places dilated tubuli, with disintegrating, purulent content. Solitary fibrous foci in the marrow.

**R 594.** Died 7 days after the last inoculation.

*Autopsy:* Bitten in the scrotum so that the left testicle is hanging out of a crustaceous mass. The lungs show several portions that are lighter and firmer than their surround-

dings. The spleen is of normal size. Slightly increased delineation in the liver. Smears from the liver contain sparse fungus cells. A couple of haemorrhages with coagulation the size of a 1-shilling coin, in the omentum.

*Microscopic examination:* In the lungs are noticed here and there perivascular and peribronchial round cell infiltrations. Furthermore, oedema and hyperaemia. The liver displays diffuse, fresh necroses, as a rule in the centre of the acini. Slight infiltration here. The picture agrees with the one in R 503, though it is not so pronounced. In the pulp of the spleen and around the follicles acute, splenic changes. No large content of blood-pigment. Numerous, fine cicatrose striae, with moderate round cell infiltrations, pervade the kidneys, and also scars with broad wedge-shape in the cortex.

**R 595.** Hardly 2 months after the fifth inoculation there was noticed cataract in the right eye, and after a further 7 weeks hyalitis oculi sinister. 2 weeks later there appeared obliquity in the carriage of the head. The rabbit was killed 7  $\frac{1}{2}$  months after the last inoculation.

*Autopsy:* Small, whitish, rather firm foci in the right lung.

*Microscopic examination:* In the lung are noticeable diffuse bronchitis, peribronchitis, together with bronchopneumonic foci without distinct granulomatous character. The spleen shows trifling haemosiderosis in solitary, swollen, reticular cells. Liver and kidneys without pathological changes. Right eye: Retinal loosening with lipid deposits in the retinal mass. In one spot in the retinal loosening a largish abscess, in whose centre is lying a large fungus colony of elongated, round, and oval cells. The lens shows within the capsule granular disintegration of the periphery with calcination. Left eye: On the whole the same changes as stated above. The fungus colony, however, shows preponderantly round and oval cells in the centre and elongated ones in the periphery. In the edge of the fungus colony a peculiar, claviform congestion of the elongated cells. The colony is surrounded by a hyaline border. The central nervous system: In some odd spots in the cortex cerebri and in the vicinity of the third ventricle, glial proliferations.

**R 596.** 3 months after the last inoculation there was palpated an abscess the size of an almond on the back, in front of a healed scratch. Hyalitis oculi dexter and cataracta oculi sinister. 2 months later some weakening of the hind-legs was noticed. Died 7 months after the last inoculation.

*Autopsy:* Subcutaneously on the back is located an abscess, sharply delineated, the size of an almond, which contains only few bacteria of subtilis type. *Pronounced cachexy.* No macroscopic changes of internal organs.

*Microscopic examination:* Heart, liver and lungs without pathological changes. The spleen shows trifling pulp haemosiderosis. In the kidneys are seen in the marrow-cortex border a diffuse interstitial fibrosis without foci or cell reaction. Right eye: Partial retinal loosening. In one retino-glial proliferation focus with lipid deposits and cholesterol crystals are seen several small abscesses, partly with necrotic disintegration and here and there also dark, coarse-grained masses (calcinations?). No certain micro-organisms. Left eye: Peripheral disintegration and calcination of the lens within the capsule. Large lipid granuloma with a central, older abscess between the partially loosened gliously proliferated retina and the chorioiden. In the abscess a large fungus colony, consisting chiefly of elongated cells with claviform swellings in the periphery. The central nervous system: Partial destruction of the pyramidal cell band, with glious proliferation here. No delimited foci. Here and there perivascular round cell infiltrations in the subcortical marrow. Freely lying in the fourth ventricle is seen a lump of exudation the size of hardly a pin's head, the lumen of it being occupied by a fungus colony with a prolific mixture of round, oval, and elongated cells. Indistinct subependymal glial proliferation of the surroundings.

**R 597.** At the third inoculation some obliquity in the carriage of the head was noticed. The last inoculation was given a week later. After another 5 weeks hyalitis oculi bilateralis, and 2 months later cataracta bilateralis, together with some weakness in the hind-legs. Killed nearly 9 months after the last inoculation.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* No appreciable changes in the lungs, liver, and spleen. The kidneys show some solitary submiliary round cell infiltrations in the cortex and striae of connective tissue lightly infiltrated with round cells. Eye (only one eye examined): Peripheral disintegration with calcination of the lens within the capsule. In the circumference of the rear bulb is noticed a mighty retino-glial proliferation, and in the destroyed retina a largish lipoid granuloma with a large abscess, in whose centre is located a minor fungus colony with preponderatingly long cells. The central nervous system: In a couple of places in the cortex cerebri are found small, fibroscicicatrose, striate foci with stratified amorphous or coarse-grained masses, which resemble those described for R 586.

**R 598.** 1 week after the second inoculation there occurred disturbance of balance and gradually progressive paresis, so that the rabbit, 7 weeks after the last inoculation, was paralytic in the hind part of the body, and showed reduced strength in the fore-legs and barrel. Died 1 week later.

*Autopsy:* *Pronounced cachexy.* Liver somewhat larger than usual, with a few white, non-raised nodules the size of up to a grain of rice. In the kidneys several white nodules the size of the point of a needle. Other internal organs without macroscopic changes.

*Microscopic examination:* Lungs without pathologic changes. The spleen shows anaemic parenchyma. Pronounced haemosiderosis in the pulp. In the liver are in some places seen groups of large and small, fresh necroses without distinct reaction in the neighbourhood. The centre of the necroses is often drenched with pus. In smears from these are seen plenty of fungus cells, some acid-fast ones with Hallberg-staining, but the majority unstained, the latter being the case also with Gram-staining. The kidneys display slight, thin, cicatrose striae through the cortex. Immediately below the capsule a small granulomatous focus rich in cells around an inflammatorily changed glomerulus. The central nervous system: In the peduncular gangliae hyperaemia and perivascular round cell infiltrations, and in some few spots small, glious foci. In one cornu ammonis are noticed the same changes, as well as an abscess the size of a poppy-seed, in whose centre are lying vast quantities of unstained fungus cells. The spinal cord without visible changes. In the meninges of the cerebellum and the medulla oblongata round cell infiltrations in a few places.

**R 599.** 7 days after the second inoculation the animal had grown lean and held its head somewhat aslant, for which reason it was not inoculated then. The last inoculation was made 7 days later. After a further 7 days disturbance of balance. Died after another 11 days.

*Autopsy:* *Pronounced cachexy.* A large accumulation of pus is seen to come from the upper part of the mediastinum and then follow the large vessels to the upper thorax apperture, where the masses of pus extend over the left half of the thorax and then descend subcutaneously on the back. Skin intact. No macroscopic changes otherwise of internal organs. The large, totally necrotic, subcutaneous abscess in smears proves to contain non-acid-fast, actinomyces-like elements and gram-negative, slender rods.

*Microscopic examination:* The spleen displays haemosiderosis. The liver is without visible changes. In the kidneys cicatrose striae in the cortex, which are fairly rich in round cells, and some odd fibrose foci in the marrow. The central nervous system: In the pedunculus cerebri on the one side and in the pons a glial focus. Otherwise hyperaemia in the central gangliae. The spinal cord without visible changes.

**R 600.** 7 days after the first and only inoculation the rabbit was considerably affected, lean, and after a further 7 days increased disturbances of balance were noticeable. 5 weeks after the inoculation weakness became manifest in the hind-legs, so that the animal after a few more days was unable to support the backpart of its body. 5 weeks later the disturbance of balance still remained, but the paresis had almost disappeared. 4 months after the inoculation hyalitis oculi bilateralis had set in, and after a further month cataracta bilateralis. After that time the rabbit began to increase in weight. It was killed 10  $\frac{1}{2}$  months after the inoculation.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* In the heart there is present a focal, slightly myocardic fibrosis. The lungs are without changes. In the liver are seen some submiliary foci but without giant cells. No necrosis. The spleen presents trifling pulp haemosiderosis. In the kidney are visible several slightly round cell infiltrated striae of connective tissue in the cortex and marrow. The right eye presents peripheric necrosis and disintegration of the lens. Retinal loosening with proliferation of glious elements. In the rear of the bulb periphery is visible an older lipoid granuloma rich in connective tissue, with abscess containing a couple of brown fungus cells. Between the pus and the capsule is located a broader zone of epithelioid cell granuloma. The left eye shows the same changes in the lens and total retinal loosening. The central nervous system: Here and there in cornua ammonis are seen minor glious striae and in some of the central gangliae partly perivascular round cell infiltrations, partly a miliary granuloma of the same appearance as the one described for R 653. Subependymally in the fourth ventricle a small accumulation of quite small, perivascular granulomata without necrosis. Furthermore, in the basis of pedunculus cerebri diffuse perivascular round cell infiltrations. Slight round cell infiltration in the mucous membranes around the pons.

**R 652.** 1 month after the last inoculation there occurred progressing disturbance of balance. 7 months later vascular injection in the sclera of the right eye. Died more than 3 months after the last inoculation.

*Autopsy:* *Cachexy.* The spleen is smaller than normal, but the internal organs otherwise without macroscopic changes.

*Microscopic examination:* In the lungs severe hyperaemia and haemorrhages, and also in one place submiliary atelectatic foci with alveolar proliferation. The liver shows severe hyperaemia and a couple of miliary, fresh necroses. In the cortex of the kidney are seen narrow, cicatrose striae and in the marrow some submiliary abscesses containing large fungus colonies with some odd acid-fast (Hallberg-stain), round and ovoid cells in the centre and elongated cells in the periphery (Pl. VII, figs. 1 and 2). Round the abscesses a fibrose inflammatory capsule (Pl. XI, fig. 1). Right eye: Just behind the zonula cinnii are seen outside the core of the lens three subretinal, miliary abscesses close to each other, one of them necrotically disintegrated. In one of the abscesses is found a fungus colony, which consists chiefly of round and ovoid forms, and in another some solitary fungus cells as well as two small groups of fairly clumsy and short, acid-fast rods. Left eye: Retinal loosening with subretinal oedema and some abscesses with large quantities of fungus cells. Just behind the corpus ciliare a quite small collection of pus on the retina. The central nervous system: Within the different parts of the brain diffuse miliary and submiliary malacies in whose surrounding is seen a glial proliferation with peculiarly strong hypertrophic astrocytes. In several places perivascular round cell infiltrations and round cell accumulations in the meninges. The cerebellum displays trifling round cell infiltrations in the meninges. In the spinal cord is found symmetrical degeneration in the pyramidal lateral tracts.

**R 653.** One month after the last inoculation there appeared moderate paresis in the barrel and hind-legs. Died 5 weeks later.

*Autopsy:* Slightly reduced flesh. The spleen larger than normal and appearing like an infection-spleen. Smears from the spleen as well as cultivation, though only on Hohn-medium, gave no result. Trifling striae on the kidneys.

*Microscopic examination:* In the lungs alveolar oedema and hyperaemia. The parenchyma of the spleen seems to be post-mortally changed with severe dissolution of the structure. Furthermore, severe phlegmonoid infiltration and fibrinous precipitation. In some few places submiliary abscesses and severe oedema. The liver, too, was post-mortally changed. In some few places submiliary, purulently permeated necrotic foci. The kidneys display in the cortex numerous narrow, radiary cicatrose striae, which only in a few spots contain round cell infiltrations. In the right eye the periphery of the lens is in globular disintegration, and in the left eye are seen slight round cell infiltrations in the chorioiden. The central nervous system: In the cortex cerebri here and there slight perivascular round cell infiltrations and trifling glious



scars. The same infiltration in the cornua ammonis, central gangliae, and subcortical marrow. In the pyramidal cell band of one cornu ammonis is found an older, miliary, malacic focus permeated by proliferating glia. In the central gangliae, near the third ventricle, a submiliary granulomatous focus with surrounding accumulations of perivascular round cells without necrosis. Plenty of degenerative changes in the central gangliae (ischaemia).

**R 654.** Died intercurrently 3 weeks after the last inoculation.

*Autopsy:* Perforation of the scrotum, so that the left testis is hanging free in its funicle. On the kidneys small scars, while the rest of the internal organs are without any macroscopic changes.

*Microscopic examination:* Lungs and liver without any pathological changes. In the spleen the pulp is seen here and there to be infiltrated with leucocytes. The kidneys present in the cortex in a couple of places fibrose foci the size of up to a grain of wheat, with trifling round cell infiltration.

**R 655.** Died intercurrently 3 weeks after the last inoculation.

*Autopsy:* Macroscopically the same findings as for R 654. No microscopic examination.

**R 656.** 5 months after the last inoculation there became manifest obliquity in the carriage of the head and some weakness in the musculature of the neck. Killed 2  $\frac{1}{2}$  months later.

*Autopsy:* Apart from a major fibrose focus in the wall of the right ventricle of the heart, no macroscopic changes of internal organs.

*Microscopic examination:* In the heart is noticed in some spots myocardial fibrosis. The lungs are without pathological changes. In the liver are seen a couple of miliary granulomata, while the spleen shows hyperaemia in the pulp and slight haemosiderosis. Miliary granulomata without giant cells and without necrosis are seen near a papilla in one of the kidneys. The central nervous system: Slight round cell infiltrations in the pia of the brain, especially around the hippocampus. In some odd places in the subcortical marrow perivascular round cell infiltrations. In the basis of pedunculus cerebri and partly also in the pia of the cerebellum, signs of oedema.

**R 657.** 1 month after the 5th inoculation hyalitis oculi bilateralis, and after another 2 months bilateral cataract. Killed about 7  $\frac{1}{2}$  months after the last inoculation.

*Autopsy:* No macroscopic changes of internal organs.

*Microscopic examination:* Heart, lungs, and kidneys without any pathological changes. The liver presents some submiliary granulomata (like R 656). In the spleen trifling haemosiderosis. In the right eye retinal loosening, as well as disintegration, and granular calcination of the edge of the lens. In the left eye is noticed, in the rear part of the bulb, within the loosened, gliously altered retina, a lipoid granuloma and in its centre a slight encapsuled abscess, which contains a large fungus colony with hyaline border (Pl. XV). The central nervous system: In the lower central gangliae, in one spot, a submiliary granulomatous focus surrounded by perivascular round cell infiltrations.

**G-p 238.** Inoculated subcutaneously in the right groin with 1.5 cc of a 35 days old glycerin bouillon culture of strain I containing fungus elements and acid-fast rods. A week later an abscess fully the size of a pea was palpated at the spot of inoculation, but this had vanished after about 2 weeks. 5 months after the inoculation the animal was killed.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-p 319.** Inoculated subcutaneously in the right groin with 1 cc of 62 days old glycerin bouillon culture of strain I containing fungus elements and acid-fast rods. Killed after 2  $\frac{1}{2}$  months.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-p 320.** Inoculated like G-p 319, but with 1.5 cc. After a week at the inoculation spot an abscess twice the size of a pea, which 3 weeks later had vanished. Killed 4 months after the inoculation.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-p 321.** Inoculated like G-p 319, but with 2 cc. Killed after 9 months.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-p 329.** Inoculated subcutaneously in the right groin with 1 cc of a 36 days old glycerin bouillon culture of strain III containing fungus elements and acid-fast rods. The animal died after 2 1/2 months.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-P 333.** Inoculated intraperitoneally with 1 cc of a 13 days old glycerin bouillon culture containing fungus elements and sparse acid-fast rods. The culture transferred from Hohn-culture which in its turn had been obtained from a kidney abscess of R 589. Killed after 3 months.

*Autopsy:* No macroscopic changes of internal organs. No microscopic examination.

**G-p 334.** Inoculated subcutaneously in the right groin with 1.5 cc of the same culture as G-p 333. Killed after 8 months.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot.

*Microscopic examination:* The lungs manifest some atelectasia, and the spleen fairly strong haemosiderosis, with some swelling of the sinus walls. The kidneys without changes.

**R 687.** Weight 1,740 gr. Inoculated with 1 cc of the same culture as G-p 333. After 1 1/2 month the rabbit showed paralysis in the hind-part of the body. Died slightly less than 2 months after the inoculation.

*Autopsy:* Weight 1,050 gr. *Strongly pronounced cachexy.* No macroscopic changes of internal organs.

*Microscopic examination:* The lungs display only some focal haemorrhages and the spleen a slight haemosiderosis. The liver shows nothing pathological. In the kidneys are seen in the cortex solitary, small calcinations of the tubular epithelium. Nothing pathological in the eyes. The central nervous system: In one pedunculus cerebri a loosely built, submiliary focus and in the marrow of the cerebellum, on one side, an older malacic, miliary focus with strong glial proliferation. Solitary, loose glial foci in the pons. (The spinal cord not examined.)

**G-p 329.** Inoculated subcutaneously in the right groin with 1.5 cc of a 7 days old glycerin bouillon culture containing fungus elements and acid-fast rods (Pl. VI, fig. 1). The culture was made from liver of R 591. After 4 weeks there was observed in the right groin a healed fistula. Killed 8 months after the inoculation.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-p 330 and 331.** Inoculated intraperitoneally with respectively 1.5 and 1 cc of the same culture as G-p 329. Killed after respectively 8 and 3 months.

*Autopsy:* No macroscopic changes of internal organs. No microscopic examination.

**R 683.** Weight 2,660 gr. Inoculated with 1.5 cc of the same culture as G-p 329. After 4 days the rabbit was seriously affected and unable to keep on its hind-legs; died next day.

*Autopsy:* Weight 2,125 gr. The lungs display on the surface stelliform haemorrhagic foci. The liver is without any changes. The kidneys are pervaded by white nodules the size of a pin's head, which in smears contain plenty of fungus cells and solitary, short, acid-fast rods.

*Microscopic examination* (only the central nervous system examined): Within different parts of the brain diffuse, miliary abscesses containing large quantities of fungus cells (Pl. VII, fig. 5 and Pl. XII, fig. 3). Moreover, plenty of perivascular infiltrations and meningitic changes. The same picture in the cerebellum and pedunculus cerebri. In the spinal cord, too, are seen diffuse abscesses with fungus cells.

**R 684.** Weight 2,350 gr. Inoculated with 2 cc 9 days old glycerin bouillon culture made from the liver of R 591 (the same culture as had been inoculated into R 683, though 2 days older). Died after 7 days.

*Autopsy:* Weight 1,590 gr. *Emaciated.* At the base of the left lung an abscess formation. The spleen fairly small. The liver with no certain changes. No foci. In the kidneys are noticed crowds of generally miliary abscesses in the cortex and marrow. In several of the abscesses are manifested purulent tubuli, and in other scentral necroses, replete with partly unstained fungus cells. Around the necroses radiary location of leucocyte granules. No distinct granulomatous zone of epithelioid cells (Pl. XI, fig. 2).

**R 685.** Weight 2,680 gr. Inoculated with the same culture as R 684, though twice, with 0.5 cc and 0.75 cc of respectively 11 and 21 days old culture. 1½ months after the second inoculation the rabbit had lost 850 gr in weight. It then displayed hyalitis oculi bilateralis. The strength of the hind-legs had deteriorated, but this passed off. Gradually increased in weight, and was killed about 9 months after the last inoculation. Bilateral cataract was then noticed.

*Autopsy:* Weight 2,860 gr. *No macroscopic changes of internal organs.*

*Microscopic examination:* Heart, lungs, and liver without changes. The kidneys manifest in the cortex diffuse, thin, fibrose striae and a few cicatrose foci with a trifling quantity of round cells.

**R 686.** Weight 3,020 gr. Inoculated with 1 cc 11 days old glycerin bouillon culture from the liver of R 591. Died after 18 days.

*Autopsy:* Weight 1,480 gr. *Emaciated.* Some small, clear, cystiform formations in the mesenterium. Spleen small. Plenty of white, slightly fibrose, non-raised foci in the kidneys. The organs otherwise without macroscopic changes.

*Microscopic examination:* The lungs and liver do not manifest anything pathological, and the spleen focal, moderate haemosiderosis. The cystiform formations in the mesenterium prove to be thin-walled cysts containing parasites, which certainly belong to the intestinal worms. In the kidneys is seen focal, purulent nephritis with dilated, purulent channels in the marrow and cortex. In some odd spots near the surface of the cortex small abscesses with a few strongly suspected, non-acid-fast fungus cells.

**G-p 315.** Inoculated subcutaneously in the right groin with an emulsion in NaCl-solution of a crushed part of the lung from R 527. Killed 2½ months later.

*Autopsy:* The spleen presents slightly enlarged Malpighian bodies. Otherwise no macroscopic changes of internal organs or at inoculation spot.

*Microscopic examination:* Lungs and liver without any changes. The spleen shows strong proliferation of the wall-cells in the sinus, which assume an epithelioid type. In this way the sinus is strongly dislocated and filled with blood. The reactionary centres in the Malpighian bodies likewise hyperplastic. No delimited granulomata.

**G-p 316.** Inoculated like the preceding one, though with a larger dose. 3 weeks later there was palpated in the right groin an abscess the size of a hazel-nut kernel, which had disappeared within 1 month. Killed 10½ months after the inoculation.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot.

*Microscopic examination:* Same discoveries as for G-p 315.

**R 575.** Weight 2,470 gr. Inoculated intravenously with 1.5 cc of an emulsion in NaCl-solution of small bits of spleen from R 527. After 6 months weak in hind-legs. Died 2 weeks later.

*Autopsy:* Weight 1,310 gr. *Severe cachexy.* Outside the temporal bone a tumour fully the size of an almond, and extending forward towards the orbita, forcing the eye-globe forward, so that a considerable protrusio bulbi sinister exists. The tumour contains a yellowish-white, caseous mass. On both the knee and elbow-joints, as well as in the joint of the left foot, are seen large, nodose, caseous deposits (Pl. XVI, figs. 3 and 4), and towards the columna vertebralis, in the thoracal region, a caseous focus larger than a pea.

In the lungs are seen diffused miliary, whitish foci, and on both apices some abscesses. The spleen is not enlarged and presents on its surface some yellowish-white tuberculo-sis-like nodules. The liver is without macroscopic changes. The kidneys display on the surface hyaline, cicatrose depressions and in the parenchyma some whitish nodules (Pl. XVI, fig. 2). The ileum and caecum appear to be strewn with sulphur-coloured foci (Pl. XVI, fig. 2).

*Microscopic examination:* The tumour on the skull seems to be a partly calcinated, caseous abscess and rather reminiscent of an old tuberculous caseous focus. In the surrounding capsulo are seen epithelioid cell granulomata with caseous necroses, which resemble to a high degree tubercles (Pl. XVII, fig. 2). In columna vertebralis and the previously mentioned joints exist caseously necrotic, osteomyelitic foci, surrounded by irregular, broad epithelioid cell borders. Diffuse fresh minute necroses in the bone marrow. Similar foci also in the periosteum. In all the said foci are seen *acid-fast bacilli of Koch's type*, especially in the peripheries of the necroses (Pl. VIII, fig. 5). In the lungs are seen diffuse haemorrhages and numerous microscopic granulomatous foci with epithelioid and round cells. No necrosis. Sparse acid-fast rods. They do not present any very large similarity to the. The spleen shows an enormous haemosiderosis and diffuse, large epithelioid cell granulomata with numerous rudimentary giant cells as well as a central necrosis with calcination. Large quantities of *acid-fast rods*. The suprarenals are without any changes. In the kidneys are seen major and minor striae rather deficient in cells, and these striae pass up through the cortex, furthermore fresh cortical granulomata of an elongated form and consisting of masses of epithelioid cells and centrally caseous necroses with *acid-fast bacilli* (Pl. XVII, fig. 1). *Great similarity with the.* Granuloma occurs also in the marrow. In the tubuli a couple of more or less purulent secretions. In the center of a necrosis is seen a glomerulus remnant and in this plenty of *acid-fast rods*. Sections from the wall of ileum show in the lymphatic tissue closely lying granulomatous foci. Numerous epithelium cavities are dilated and filled with pus. In one such cavity are encountered a couple of oval, pale yellowish-brown formations with cuticular shell and homogeneously stained content, partly with amorphous, granular masses. (Intestinal parasite?) In the granulomata there are moderate quantities of *acid-fast rods*, partly in small groups. The brain: In solitary spots in the cortex are seen small, glious scars and perivascular, minute granulomata. In the central gangliae of one side an older abscess surrounded by perivascular round cell infiltrations. In the centre of the abscess a necrosis of caseous appearance with a remarkably large number of *acid-fast rods*, partly diffuse, partly in bundles or balls (Pl. VIII, fig. 4). The cerebellum is without visible changes.

**R 584.** Weight 2,650 gr. Inoculated like R 575, though with 1 cc. Killed after 3 months.

*Autopsy:* In the central lobe of the right lung a hyaline portion the shape of a clover-leaf. The spleen of normal size, with several white nodules the size of a pin's head on the surface. Otherwise no organic changes visible.

*Microscopic examination:* In the lung are not seen any granulomatous foci, but here and there epithelioid proliferations in the alveolar walls. No necrosis. The spleen presents diffuse epithelioid cell granulomata with numerous rudimentary giant cell formations, and in some of the foci commencing caseous necrosis. No manifest haemosiderosis. The liver rich in glycogene, but otherwise nothing remarkable.

**G-p 307.** Inoculated like R 584, though subcutaneously in the right groin. 3 weeks after inoculation there was felt at the spot where the injection was made in the right groin an abscess the size of a hazel-nut kernel.

*Autopsy:* No macroscopic changes of internal organs. In the right groin an abscess which in smears proves to contain *acid-fast rods*.

*Microscopic examination:* The spleen presents anaemic pulp and sinus content rich in leucocytes, and also here and there plasmatic cells. No haemosiderosis. Liver and kidneys without changes. The abscess in the groin seems to be encapsuled by a membrano with the appearance of granulomatous tissue rich in cells. In one spot in the same an indistinctly delimited, rounded epithelioid cell granuloma, without giant cells or necrosis.

**G-p 324.** Subcutaneous inoculation in the right groin with 0.5 mg of 57 days old culture on Hohn-medium of acid-fast rods originating from the spleen of R 527. 10 days later was palpated at the spot of injection an abscess the size of fully a hazel-nut, which after another 3 weeks fistulated. Killed 9 months after the inoculation.

*Autopsy:* No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

**G-p 325.** Inoculated like G-p 324, though with 0.25 mg. Abscess in the right groin and fistulation as in the above-mentioned animal. Killed 3 1/2 months after inoculation.

*Autopsy:* In the right groin are seen two abscesses the size of a pea, and on the right side of the arteria iliaca dexter a lymph gland the size of a hazel-nut kernel which had partly turned into an abscess.

*Microscopic examination:* In the lungs indicated granulomatous foci without necrosis. The liver without any changes. In the lymph gland is seen an epithelioid cell granuloma with central abscess formation, but without necrosis. Sparse *acid-fast rods*. The lumbar abscess is surrounded by a narrow edge of epithelioid cells. In some spots necrosis in the pus. Rather plenty of *acid-fast rods*, single ones, in masses and under phagocytosis.

**R 689.** Weight 2,630 gr. Inoculated with 1 cc emulsion of pus from an osteomyelitic focus of R 575 (a speck of pus the size of a pea was emulsified in 10 cc NaCl-solution). Died 1 1/2 month later.

*Autopsy:* Weight 1,610 gr. *Severe cachexy*. The pulmonary parenchyma poorly air-conducting with a few white foci the size of a pin's head. The spleen greatly enlarged (Pl. XVIII, fig. 1), 10 cm long and pervaded by white nodules the size of a pin's head. The liver, too, is enlarged and presents the same foci as in the spleen, though not in such large numbers. On both kidneys diffuse nodules of the same appearance as in the spleen. In the wall of caecum are distinguished a few whitish foci.

*Microscopic examination:* In the pulmonary parenchyma severely desquamative catarrh and oedema, as well as numerous epithelioid cell granulomata surrounded by lymphocytic casings. In some odd granulomata central caseous necrosis. *Looks like tuberculous changes*. Moderate quantity of *acid-fast rods*, partly diffuse and partly in groups. The spleen is plentifully pervaded by large and partly confluent caseous necroses, surrounded by thinner or broader epithelioid cell borders. Here and there typical Langhans' giant cells. Histologically the changes are on the whole identical with *tuberculous changes* (Pl. XIX). The necroses are pervaded by enormous quantities of *acid-fast bacilli*, especially in the periphery, where they are lying partly diffuse and partly in large balls, and also partly in macrophagi and in the Langhans' giant cells (Pl. VIII, fig. 8). The liver presents masses of typical military tubercles, the majority with central caseous necrosis and with diffuse Langhans' giant cells. Plenty of *acid-fast bacilli*, diffuse and in groups or balls. In the cortex of the kidney are visible diffuse submiliary granulomata without caseous necrosis or giant cells, partly built up of epithelioid cells. Here and there *acid-fast rods*.

**R 690.** Weight 2,725 gr. Inoculated like R 689, though with 1.5 cc. Died after 2 months.

**Autopsy:** Weight 1,300 gr. *Severe cachexy.* The lungs are pervaded by major and minor caseous foci. In the spleen and liver plenty of white nodules hardly the size of a pin's head (Pl. XVIII, fig. 2). In the kidneys a small number of foci. The caecum swollen and pervaded by whitish nodules.

**Microscopic examination:** As a rule, the lungs display in the parenchyma, especially below the pleural surface, *miliary and major tubercles*, with caseous necroses and purulent admixtures. Diffuse Langhans' giant cells in the surroundings. A moderate quantity of *acid-fast rods*. Histologically judged, the process is of recent origin (Pl. XX). In the spleen are visible severe pulp haemosideroses as well as numerous *miliary tubercles* with central caseous necroses and Langhans' giant cells. Severe reticulo-endothelial hyperplasia in the pulp. Plenty of *acid-fast rods*, singly and in accumulations. The liver presents mainly the same picture as in R 689, but a more pronounced necrotic tendency. Several of the necroses hyalinely encapsuled or metamorphosed, respectively (Pl. XXI, fig. 1). *Acid-fast rods* as in the spleen. In the cortex of the kidney fairly numerous miliary and submiliary granulomata, the larger ones with central caseous necrosis. A moderate quantity of diffuse *acid-fast rods*.

**G-p 380.** Inoculated like R 689, though intraperitoneally with 1 cc. Weight then 425 gr. Died after about 5 months.

**Autopsy:** Lean (weight 340 gr.). Internal organs hyperaemic but otherwise without macroscopic changes.

**Microscopic examination:** The lungs and liver present intensive stasis and the spleen is rich in cells, moderately thickened sinus walls and moderate haemosiderosis. Otherwise no pathological changes.

**G-p 381.** Inoculated like G-p 380, though subcutaneously in the right groin. Still alive 7 months later.

**G-p 383.** Weight 425 gr. Inoculated like G-p 381, though with 1.5 cc. 14 days after inoculation there was palpated in the right groin an abscess the size of a hazel-nut kernel, which remained when the animal died within 5 months of the inoculation.

**Autopsy:** Weight 370 gr. Lean. In the right groin is visible an abscess the size of a hazel-nut, with yellowish-white pus. A slightly enlarged lymph gland on the right arteria iliaca. A slightly increased quantity of moisture in the belly. In the mesenterium two whitish formations the size of a pea. Otherwise nothing pathological visible. The bladder contains a concrement the size of a pea and with a rough surface.

**Microscopic examination:** Lungs, liver, and kidneys without any changes. In the bladder trifling chronic cystitis. The formations in the mesenterium prove to be lymph glands with lymphatic stasis. The lymph gland near the arteria iliaca presents slight, chronic adenitis, but otherwise nothing pathological. In the right groin a chronic abscess, containing fairly sparse, *acid-fast rods*, and surrounded by an abscess membrane with numerous epithelioid cells.

**R 665.** Inoculation of 0.2 mg of Hohn-culture derived from an osteomyelitic focus in R 575. Died 3 weeks after the inoculation.

**Autopsy:** Lungs, liver, and spleen present a fine-grained surface, but no macroscopic foci. The spleen is enlarged.

**Microscopic examination:** In the lungs numerous epithelioid cell granulomata. No caseous necrosis and no distinct formation of giant cells. Plenty of *acid-fast rods*, diffused and in groups. In the liver lots of quite small epithelioid granulomata, diffusely strewn about and without caseous necrosis. Rudimentary giant cells here and there. No manifest histological similarity to the. *Uncommonly plenty of acid-fast rods*, largely in bundles and balls. The parenchyma of the spleen is for the greater part replaced by generally small epithelioid cell granulomata, often arranged as thick casings around the Malpighian bodies. No caseous necrosis and no manifest giant cell formation. *Remarkably plenty of acid-fast rods*, mostly in bundles and balls. The kidney presents acute inflammatory perirenal oedema. In the cortex of one kidney is found

a fibrotic focus about the size of a pea, within which the glomeruli are mostly preserved, but the ducts atrophic. No acid-fast rods.

**R 542.** Inoculated with 0.2 mg of Hohn-culture from the spleen of R 689. Died after 16 days. No appreciable change in weight.

*Autopsy:* Spleen enlarged. Otherwise no macroscopic changes of internal organs.

*Microscopic examination:* The lungs display indications of bronchopneumonic infiltrations and minor atelectatic foci. Moderate quantity of *acid-fast rods*. In the liver plenty of granulomatous foci, which are located pronouncedly periportal, respectively in the periphery of the acini. Some odd minor giant cells but no caseous necrosis (Pl. XXI, fig. 2). Plenty of *acid-fast rods*, largely lying in big heaps and balls. The spleen displays diffuse reticular cell proliferation with sharply delimited epithelioid granulomatous foci, with here and there indicated necroses. Trifling pulp haemosiderosis. *Acid-fast rods* as in the liver. The kidneys show solitary fibrotic cortical striae infiltrated by round cells, partly reaching down into the marrow. In one place in a pyramid a submiliary granulomatous focus of epithelioid cells, surrounded by round cell infiltrations. No necrosis. Some groups of *acid-fast rods*, inter alia in a couple of glomeruli.

**G-p 384.** Inoculated subcutaneously in the right groin with a bit of the spleen quite the size of a pea from R 690, and emulsified in NaCl-solution. After about 2 weeks there was palpated a fluctuating abscess almost the size of a walnut in the right groin. 3 months after the inoculation the animal was treated on the back with barium sulphide for the purpose of getting a surface free from hair for tuberculin tests, and the guinea-pig died within 14 hours.

*Autopsy:* In the right groin an abscess the size of a hazel-nut with large quantities of pus that has penetrated into the femoral muscles. No distinct organic changes.

*Microscopic examination:* The abscess in the groin is chronic with serious disintegration of the pus. Epithelioid cells of no very distinct character are seen in the surrounding abscess membrane. In the abscess rather plenty of *acid-fast rods*, most plentiful where the disintegration of the cells is greatest. The lymph gland near the arteria iliaca shows numerous submiliary epithelioid cell granulomata without caseous necrosis and without acid-fast rods. The spleen presents strong reticular cell proliferation in the pulp, but no delimited granulomata. Trifling haemosiderosis. In the liver is seen, in conjunction with a portal cord, a trifling epithelioid cell focus.

**G-p 385.** Inoculated like G-p 384. After two weeks there became visible in the right groin an abscess the size of a hazel-nut, which fistulated a week later. The animal is alive and well 5 months after the inoculation.

**R 543.** Weight 2,700 gr. Inoculated with 0.1 mg of Löwenstein-culture derived from the liver of R 690. Died 5 weeks after the inoculation.

*Autopsy:* Weight 2,050 gr. Enlargement of the spleen but otherwise no distinct organic changes.

*Microscopic examination:* In the lung numerous miliary and submiliary tubercles. Pronounced histological similarity to tuberculosis. Moderate quantity of diffuse *acid-fast rods*. In the liver vast quantities of caseous necroses, partly confluent and surrounded by loose, broad zones of epithelioid cells. Only solitary giant cells of Langhans' type. Great histological similarity to tuberculosis. Moderate quantity of *acid-fast rods*, here and there plentiful. The spleen presents numerous miliary epithelioid cell granulomata, located especially around the arteries, some of them with central caseous necrosis. Solitary Langhans' giant cells. Great similarity to *ibc*. Findings of *acid-fast rods* as in the liver. The kidneys do not show anything worth noting.

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Undritz examined the blood from the parents in both these cases with the following results: In Stahel's case both the parents proved to be »partial carriers», the father of the usual type and the mother of the type described by Leitner where the leucocytes have coarse chromatin net and 3—5 round segments with thin linking threads. — In Zündel's case, the father at least was »partial carriers».

Nevertheless the hereditary conditions are not made clear by these finds, nor would Undritz suggest it. It should be strongly emphasised that »partial carriers» are not considered as bearers of the Pelger gen.

As stated, none of the relatives of our Pelger cases were »partial carriers», therefore including the parents of our case No. 2 — and this despite very careful examination of their blood. — The patient had no children.

Possibly in this case there may be question of a Pelger type that is new as regards occurrence. Illegitimate paternity might, however, provide the explanation. This possibility was considered to be excluded in Zündel's case.

Pelger-Huët's anomaly of the nuclei of the leucocytes, false shift to the left, has considerable theoretical interest, as has been seen. Undoubtedly, however, the practical importance is also great. If the anomaly is diagnosed, Pelger individuals would then of course be spared many complicated examinations and possibly also more or less risky or financially burdensome therapeutic interventions. — Consequently the false shift to the left requires to be known to the greatest extent possible.

At Södersjukhuset we have now had in the course of little more than a year 4 cases of false shift to the left. This indicates that this anomaly is not so uncommon. If a watch is kept out for the characteristic feature of the blood picture, these cases will probably be diagnosed to an ever greater extent.

### Summary.

Pelger-Huët's anomaly of the nuclei of the leucocytes is characterised by a noticeable contrast between the immature form of the leucocyte nuclei and their mature structure. The nucleus seems small in relation to the cytoplasm. Dumb-bell or peanut shape in these is pathognomonic, but not the glass-eye form. It is exceptional for more than 2 segments to be formed. It would



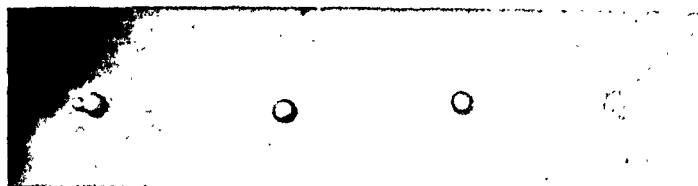
*Plate I.*

Fig. 1. Microphotograph. Strain III. Four fungus cells after 12 hours' growth at living-room temperature on the surface of maltose agar. The outermost cells show commencing budding. Magnification, 440 diameters.

Figs. 2—6. Show the continued propagation of the fungus cells by budding. Microphotographs taken after respectively 18, 19, 22  $\frac{1}{2}$ , 26, and 36 hours' growth at living-room temperature. Magnification, 440 diameters.

Figs.

1



2



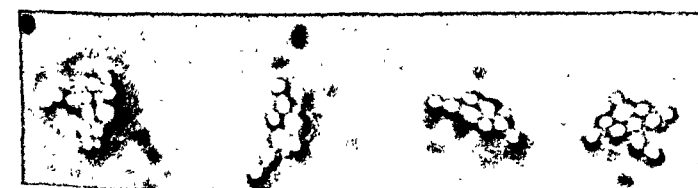
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4



5



6



*Plate II.*

Fig. 1. Microphotograph. Strain II. Part of culture on the surface of ordinary agar plate. Round, ovoid, and elongated fungus cells. Magnification, 660 diameters.

Fig. 2. Microphotograph. Strain V. Part of a culture inside an ordinary agar plate after 24 hours' growth at 37° C. Four full-grown colonies and some groups of fungus cells commencing to form a colony. Magnification, 230 diameters.

Fig. 3. Microphotograph. Strain II. Part of a culture inside an ordinary agar plate after 24 hours' growth at 37° C. In the centre a fungus colony from which issue tendrils of mycelial branches with buds. Furthermore, some more evenly outlined colonies of round and ovoid cells. Magnification, 230 diameters.



Fig. 1.



Fig. 2.



Fig. 3.

### Plate III.

(Figs. 1 and 2 are reproduced from a paper (99) by Reenstierna.)

Fig. 1 illustrates a mycotic non-acid-fast micro-organism isolated by Reenstierna in 1912 from the blood of a patient suffering from nodular leprosy in the acute stage. Growing in a special fluid medium it formed, besides solitary, large, prickly cells (one is seen at the top of the figure) chiefly long chains whose links were partly like small yeasts. Fig. 2 shows how some links have changed into acid-fastness. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 3. Strain I. Smear from 15 hours old culture on ordinary agar plate, illustrating the common appearance of fresh fungus cells. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

(Figs. 4—11. Smears from the bottom of one and the same glycerin bouillon culture, originating from a single cell of the fungus.)

Fig. 4. Smear from the culture after 36 days at 37° C. In between the fungus cells are seen blue granules with a reddish tint, partly lying in faintly blue-stained disintegrated fungus cells. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 5 and 6. Smear from the culture after 45 days. In fig. 5 are seen, alongside of some fungus cells, non-acid-fast rods, of which a couple with acid-fast granules at one end, and in fig. 6 an agglomeration of non-acid-fast and acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 7—11. Smears from the culture after 36 days. There are seen acid-fast filaments, which are interwoven with the fungus cells (fig. 7), acid-fast, granular rods of the same appearance as Koch's bacilli (fig. 8), as well as amongst the fungus cells acid-fast, dense accumulations of at least at the periphery clearly noticeable rods (figs. 9 and 10). Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 11. Smear from the culture after 45 days. Gram-positive and -negative fungus cells, of which latter one is much larger than the others. In the upper part of the fig. is seen a gram-positive net-work, which with Ziehl-Neelsen stain is acid-fast and appears like that in fig. 7. Stain: Gram. Magnification, 1,000 diameters.

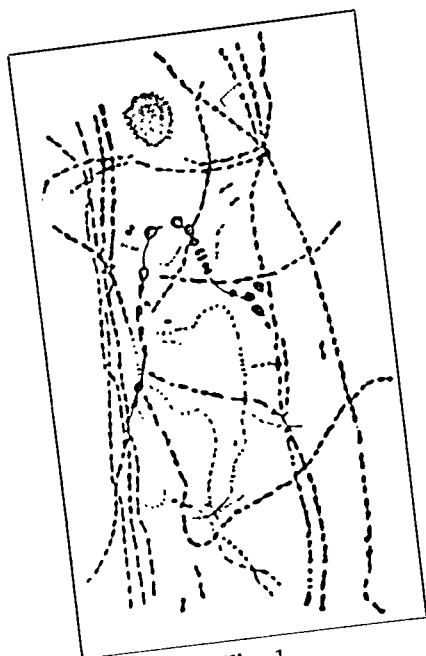


Fig. 1.

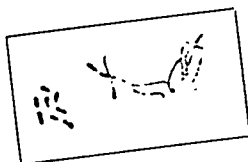


Fig. 2.

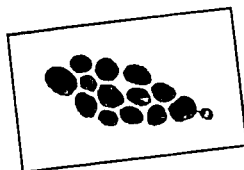


Fig. 3.



Fig. 6.



Fig. 7.

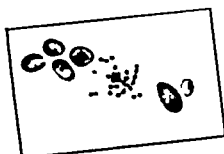


Fig. 4.

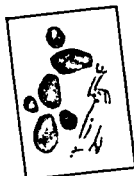


Fig. 5.

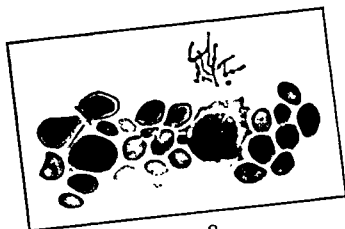


Fig. 8.

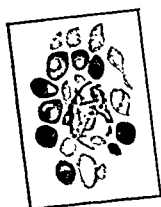


Fig. 9.



Fig. 10.

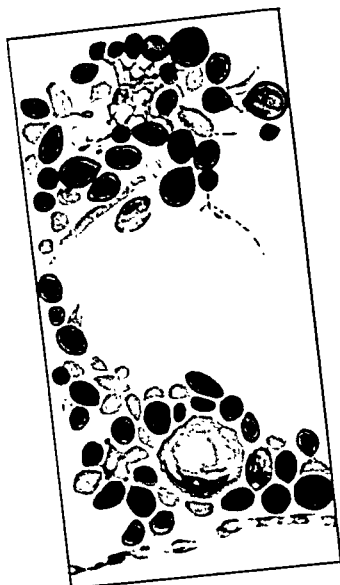


Fig. 11.

#### Plate IV.

Fig. 1. Smear from the same glycerin bouillon culture as in Pl. III, figs. 4—11, 45 days old. Acid-fast rods and filiform elements, of which one of the latter apparently issues from a fungus cell. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 2. Strain I. Smear from a 62 days old glycerin bouillon culture, which for 30 days has been growing at 37° C and 32 days at living-room temperature. Amongst the acid-fast and non-acid-fast fungus cells, some of which are faintly stained, is seen a vast quantity of granular acid-fast rods. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Figs. 3 and 4. Strain III. Smears from a glycerin bouillon culture after respectively 9 and 13 days' growth at 37° C. In both figures are observed around the acid-fast and non-acid-fast fungus cells amorphous, yellowish-brown masses containing differentiated rods, partly of the same yellowish-brown colour, partly blue (acid-fast). In fig. 3 granular and circular elements are seen forming the outline of light-blue stained, more or less disintegrated fungus cells. In the lower part of fig. 4 are observed some very large, cystiform, brown formations, probably swollen fungus cells in the course of disintegration. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

(Figs. 5 and 6 are reproduced from a paper (41) by Gullberg. They illustrate smears from glycerin bouillon cultures originating from single cells of two of Gullberg's fungus strains of yeast-oidium type.)

Fig. 5. Smear from a 13 days old glycerin bouillon culture of the one fungus strain isolated from the sputum of a patient suffering from pulmonary tuberculosis. There are seen non-acid-fast fungus elements, and in an amorphous mass small granules and small rods, of which a few are acid-fast. The red elements are intermingled in a peculiar manner with the blue components of the amorphous mass. Stain: Fontes. Magnification, 1,000 diameters.

Fig. 6. Smear from a 16 days old glycerin bouillon culture of the other fungus strain isolated from a patient with fever and erythema nodosum in his history. In this figure is seen a large, round formation, whose periphery consists of a wreath of ovoid fungus cells, while its centre is occupied by a net-work of blue filaments, in which latter there are acid-fast rods precisely like the bacilli of Koch. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

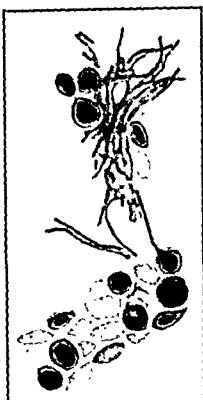


Fig. 1.



Fig. 4.

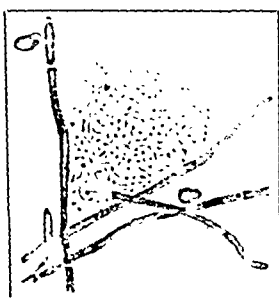


Fig. 5.

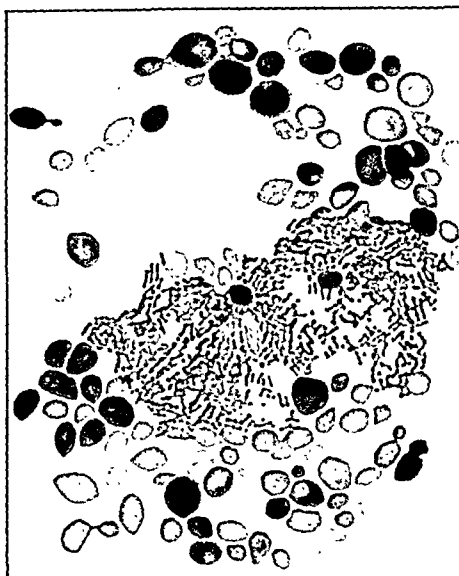


Fig. 2.

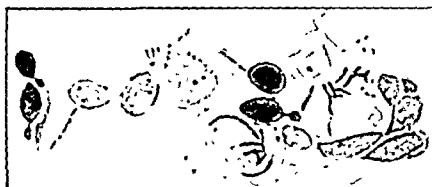


Fig. 3.



Fig. 6.



Fig. 1. Microphotograph. Strain III. Smear from a 42 days old glycerin bouillon culture (with 15 per cent glycerin instead of the ordinary 3 per cent). Amongst the fungus cells are seen diffuse acid-fast rods precisely like the bacilli of Koch. Stain: Hallberg (Nachtblau-pyronin). Magnification, 800 diameters.

Fig. 2. Smear from the same culture as in fig. 1, showing a bundle of acid-fast rods alongside acid-fast and non-acid-fast fungus cells. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 3. Strain I. Smear from a 35 days old glycerin bouillon culture. Blue and red fungus cells and three large, yellowish, peculiar corpuscles covered with red prickles. They agree in appearance with the pine-cone-shaped formations which by Hallberg have been proved in fungus cultures and sputa from patients suffering from pulmonary tuberculosis, and also in the tissues from Schaumann's disease (43). Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

(Figs. 4—12. Smears from a glycerin bouillon culture originating from the liver of a rabbit inoculated with fungus culture. This rabbit, R 591, which was inoculated intravenously five times with glycerin bouillon culture of strain II, died 5 weeks after the last inoculation, when, inter alia, it manifested endocarditis verrucosa mycotica (Pl. IX, fig. 1), as well as necroses in the lungs and liver (Pl. IX, fig. 2). A piece of the liver was transferred into glycerin bouillon, where, after one day, there was obtained growth of fungus, and within 9 days acid-fast rods.)

Figs. 4—6. Smear from the culture after 9 days. In some of the fungus cells are noticed reddish "foci". Fig. 4 illustrates acid-fast rods arranged in a bunch which in size and outline shows similarity with an ovoid fungus cell and lying like a continuation of two such. Figs. 5 and 6 show amongst the fungus cells acid-fast elements arranged in bundles of ovoid form. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 7 and 8. Smear from the culture after 7 days. Fig. 7 shows amongst the fungus cells a formation of intercrossing acid-fast rods. This formation agrees in size and shape with an ovoid fungus cell. In fig. 8 is seen how from a non-acid-fast mycelial branch seems to issue a formation of acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 9. The same smear as in figs. 4—6. Amongst the fungus cells there is a small ball of fairly sparsely lying acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 10 and 11. The same smear as in figs. 7 and 8. Fig. 10 shows amongst long fungus cells, partly in a state of disintegration, a large ball of densely packed acid-fast rods, and fig. 11 two peculiar, acid-fast formations (no doubt fungus elements). Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 12. The same smear as in fig. 9. This fig. illustrates in an agglomeration of ovoid, non-acid-fast fungus cells of ordinary size, a larger acid-fast ovoid cell, ostensibly consisting of closely packed acid-fast elements. At the upper end of this formation there projects a rod-shaped "handle". Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

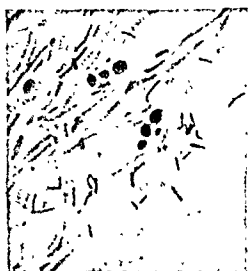


Fig. 1.

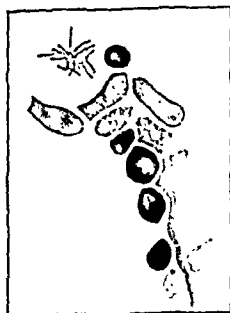


Fig. 2.

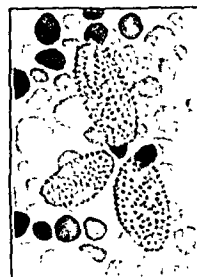


Fig. 3.



Fig. 4.

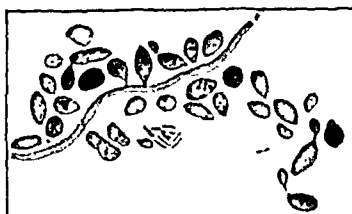


Fig. 5.



Fig. 6.



Fig. 7.

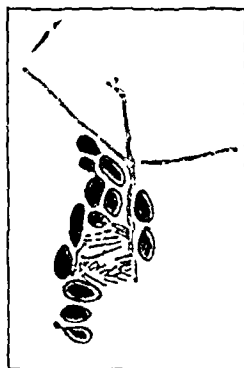


Fig. 9.

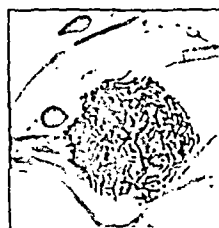


Fig. 10.

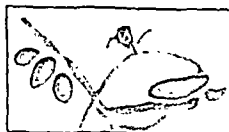


Fig. 8.

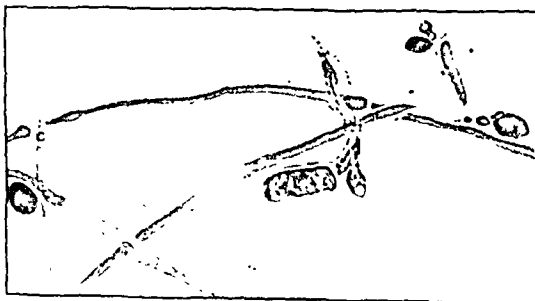


Fig. 11.



Fig. 12.

*Plate VI.*

Fig. 1. Smear from the same glycerin bouillon culture as in Pl. V, figs. 4—12, after 7 days. Non-acid-fast fungus elements, particularly mycelial branches, some of which manifest disintegration with surrounding amorphous, granular masses. Three large accumulations of acid-fast rods with the appearance of Koch's bacilli. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 2. Smear from a 53 days old culture on Hohn-medium, originating from a renal abscess from rabbit R 589, which had been inoculated intravenously with 1 cc of glycerin bouillon culture of strain II and died 7 days after inoculation. (Smears from renal abscesses showed fungus cells and very sparse, short acid-fast rods.) This fig. shows acid-fast and almost unstained fungus cells, and also acid-fast granules and short rods. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 3. Smear from renal abscess of a rabbit, which for the preparation of immune serum had begun to be inoculated with a mixture of the five fungus strains, but died 3 days after the second inoculation. An accumulation of granular and rod-shaped, acid-fast elements. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

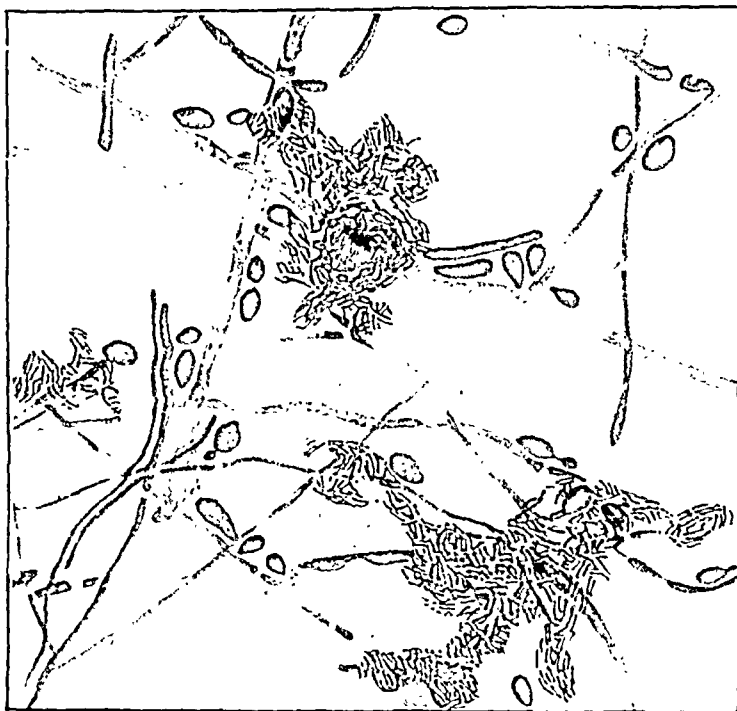


Fig. 1.



Fig. 2.



Fig. 3.

### *Plate VII.*

Figs. 1 and 2. Microphotographs of smears from a renal abscess (Pl. XI, fig. 1) of a rabbit, R 652, which had been inoculated five times with glycerin bouillon culture of fungus strain IV, and died hardly 3 1/2 months after the last inoculation. Fig. 1 illustrates fungus cells of which several have not been stainable at all or only very slightly so. Stain: Gram. Magnification, 1,000 diameters. Fig. 2 shows solitary acid-fast fungus cells, while the bulk are unstained. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

Fig. 3. Part of section of a subretinal abscess in the eye of the above-mentioned rabbit. A bunch of fairly clumsy and somewhat short, acid-fast rods, is seen. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 4. Part of a section (Pl. XII, fig. 2) from the brain of a rabbit, R 561, which had been inoculated intravenously three times with glycerin bouillon culture of strain I and died 3 days after the last inoculation. In an abscess is seen a chain of non-acid-fast, double-contoured fungus cells. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 5. Part of section from the brain (Pl. XII, fig. 3) of a rabbit, R 683, which had been inoculated intravenously with a 7 days old glycerin bouillon culture containing fungus elements and acid-fast rods. This culture had been made from the liver of a rabbit, R 591 (Pl. V, figs. 4—12 and Pl. VI, fig. 1). In an abscess is seen an agglomeration of slightly violet-stained fungus cells, some of which are plentifully provided with granules, which impart to the cells a certain likeness with the pine-cone-forms described by Hallberg. Stain: Nissl. Magnification, 1,000 diameters.



Fig. 1.



Fig. 2.

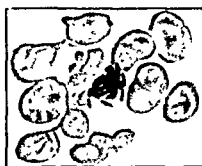


Fig. 3.

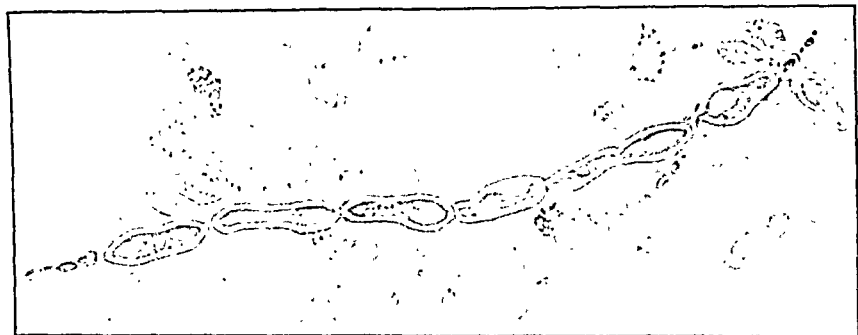


Fig. 4.

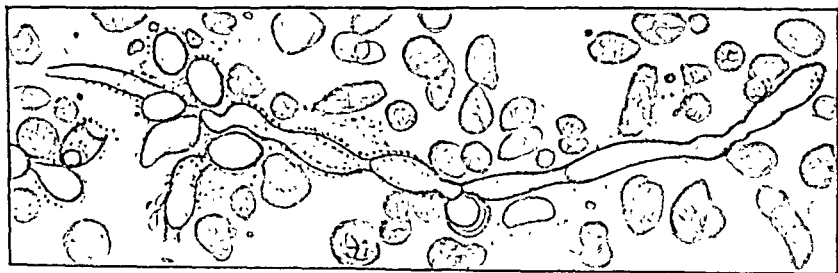


Fig. 5.

*Plate VIII.*

Fig. 1. Microphotograph. Smear from the right lung of a rabbit, R 527, inoculated intravenously twice with glycerin bouillon culture of strain II. The animal was killed after about 6 months. The autopsy showed macroscopically tuberculous changes in the lungs, liver, and spleen. Histologically, there were observed in the lungs epithelioid cell granulomata with caseous necroses. The picture reminded one not so little of tuberculosis. This fig. shows acid-fast rods of the same appearance as typical Koch's bacilli. Stain: Hallberg (Nachtblau-carbol-fuchsin). Magnification, 1,000 diameters.

Figs. 2 and 3. Smear from the spleen of R 527. Histologically, the spleen showed granulomatous foci, some of them with polynuclear giant cells but without necrosis. Foci of the same appearance were also found in the liver (Pl. XVI, fig. 1). The figs. illustrate groups of acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 4. From a section of the brain of a rabbit, R 575, inoculated intravenously with a suspension in NaCl-solution from the spleen of R 527. R 575 died in an extremely cachectic condition 6½ months after the inoculation, and displayed changes in lungs, liver, spleen, kidneys, intestine, and skeleton, some of them reminiscent of tuberculosis (Pl. XVI, fig. 2—4, Pl. XVII). This fig. shows a small portion of a cerebral abscess with partly diffuse bundles of acidfast elements, arranged similar to globi the same as leprosy bacilli, and partly a largish ball, composed of minor balls or tufts of acid-fast elements, so that the whole has a fan-like appearance. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

Fig. 5. Smear from an osteomyelitic focus of R 575. Acid-fast rods, partly in a bundle, and partly some diffuse ones. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 6 and 7. Smear from an abscess in the right groin of a guinea-pig, G-p 325, inoculated subcutaneously in the right groin with 0.25 cc of a 57 days old Hohn-culture, originating from the spleen of R 527. G-p 325 was killed 3½ months after the inoculation. Fig. 6 shows diffuse, acid-fast rods and fig. 7 a phagocytosed group of such. The phagocyte cell is in a state of dissolution. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 8. Part of a section from the spleen of a rabbit, R 689, inoculated with pus from an osteomyelitic focus of R 575. R 689 died after 1½ month severely cachectic and with organic changes as in tuberculosis (Pl. XVIII, fig. 1, and Pl. XIX). This figure illustrates acid-fast rods, mostly in large, fan-shaped agglomerations. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.

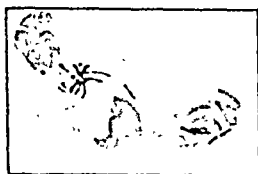


Fig. 6.



Fig. 7.



Fig. 8.



*Plate IX.*

Fig. 1. R 591. (See explanation of Pl. V, figs. 4—12.) The border portion of a mycotic focus the size of a pea on a chorda tendinea in the right cardiac chamber. Stain: Hallberg (Nachtblau-neutral-red; decoloration with propylic alcohol). Microphotograph. Magnification, 340 diameters.

Fig. 2. R 591. Two mycotic-necrotic foci in the liver. (A piece of the liver was transferred into glycerin bouillon, where, after the lapse of one day, fungus elements, and after 9 days, acid-fast rods, appeared (Pl. V, figs. 4—12, Pl. VI, fig. 1). Stain: Haem. v. Gieson. Microphotograph. Magnification, 29 diameters.

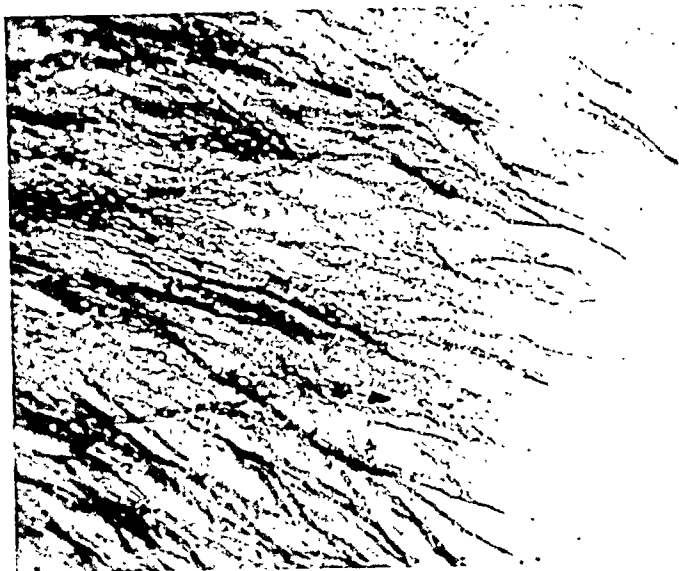


Fig. 1.



Fig. 2.

*Plate X.*

Figs. 1 and 2. R 587. Inoculated intravenously five times with glycerin bouillon culture of fungus strain I, died  $2\frac{1}{2}$  months after the last inoculation with pronounced cachexy. Fig. 1 shows necrotic abscesses in the liver. Stain: Haem. v. Gieson. Microphotograph. Magnification, 95 diameters. Fig. 2 illustrates a larger hepatic gall-duct containing fungus cells, some of which are unstained. Stain: Hallberg (Nachtblau-neutral-red). Microphotograph. Magnification, 1,000 diameters.

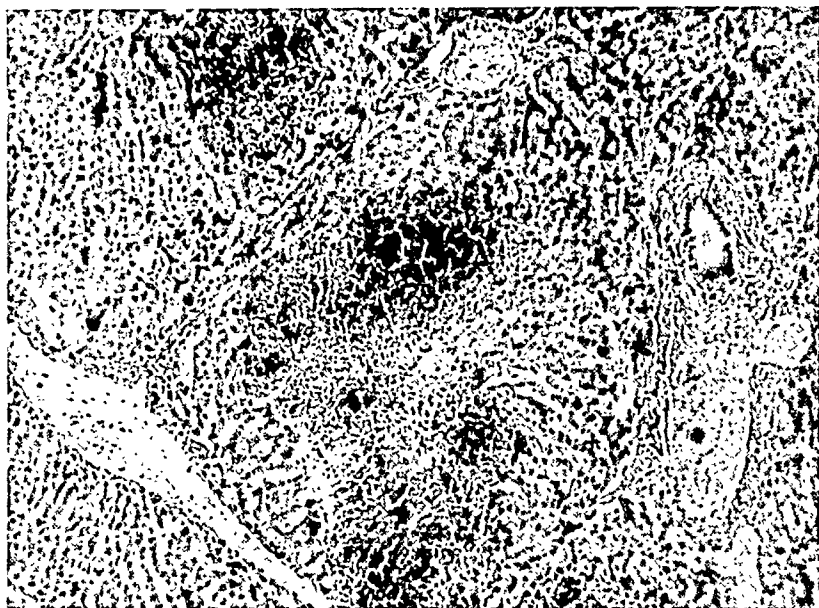


Fig. 1.

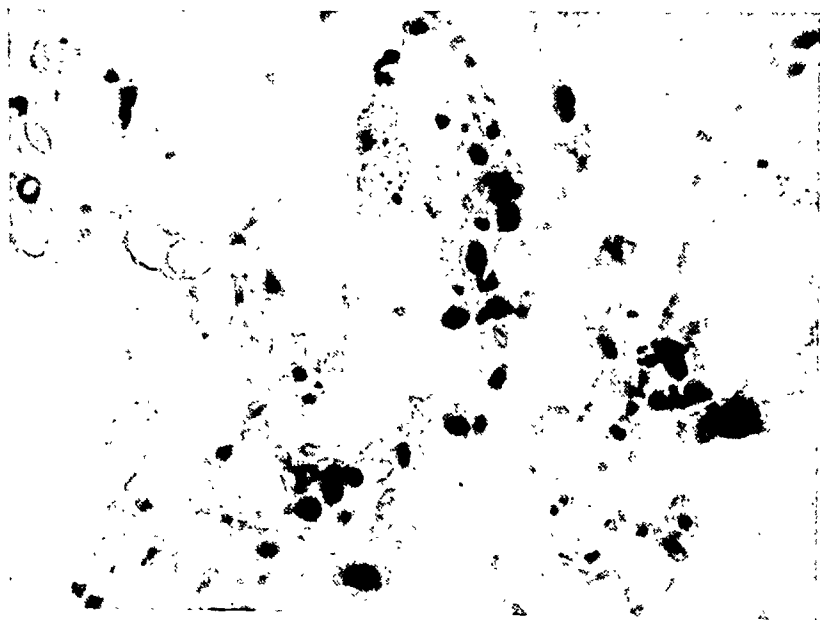


Fig. 2.

*Plate XI.*

Fig. 1. R 652. Inoculated intravenously five times with glycerin bouillon culture of strain IV, died cachectic 3 months after the last inoculation. This fig. shows in the marrow of the kidney two older abscesses with disintegrating pus. (In the pus plenty of fungus cells, as Pl. VII, figs. 1 and 2, show.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 75 diameters.

Fig. 2. R 684. Inoculated intravenously with glycerin bouillon culture containing fungus elements and acid-fast rods. This culture had been made from the liver of R 591. R 684 died after 8 days. This fig. illustrates miliary, fresh abscesses in the border between the medulla and cortex of a kidney. (Analogous pictures have been obtained also after inoculation with merely fungus cells.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 40 diameters.

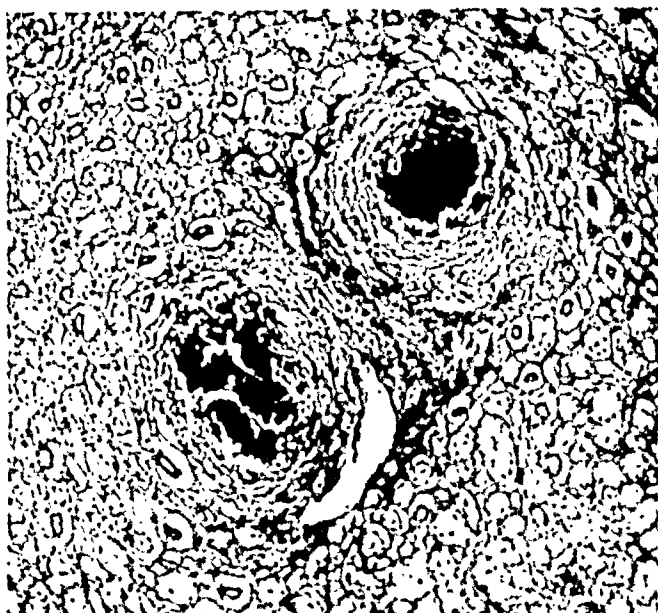


Fig. 1.

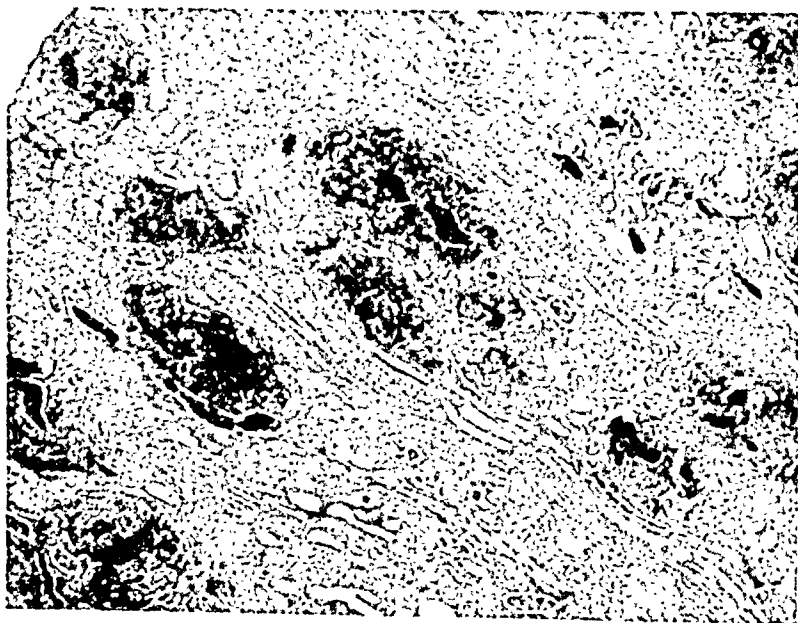


Fig. 2.



age of several measurements repeated on the same subjects in different days.

The residual air changes may be considered as due, in part at least, to variations in the extension of the functioning alveolar fields and in the blood filling of the lungs. Those changes of residual air would induce a corresponding variation on the resting respiratory level, if a concomitant modification of the reserve air does not take place. Obviously we are dealing here with the constancy or inconstancy of the resting expiratory level of the thorax, which according to Wolff (11) shows a tendency to become fixer in the pulmonary tuberculosis. The point is not well established yet, and it will require further studies. It must not be forgotten, however, that in tuberculous patients an occlusion of a bronchiole by secretions could temporarily exclude from the active gas circulation a cavity or a number of alveoli with the consequent modification of the residual air volume.

Before going further in the discussion it would be interesting to have similar data from normal subjects.

Such variability of the residual air volumes must not be disregarded in studying the changes produced on the lung volume by the surgical treatment of cancer or tuberculosis of the lungs.

For judging the significance of a change of the mean value of the functional residual air, in pulmonary tuberculous patients, measured by duplicate and with the method employed by us, the S. D. of  $\pm 5.4$  per cent of the volume measured should be used.

For residual air the S. D. of  $\pm 9.1$  per cent of its volume or  $\pm 6.3$  per cent of the volume of the functional residual air should be used.

The analysis of the 296 duplicate measurements of functional residual air made on pulmonary tuberculosis shows, that the results secured with our technic agree fairly well with those obtained by Cournand et al. with their open circuit method.

The variability of the average of our duplicate measurements of functional residual air as single determinations are much lower than that obtained by Birath, S. D.  $\pm 48$  against  $\pm 89$  ml. The greater volumes of his series, which determinations were made in sitting position, could perhaps account for the difference.

S. D. of single determinations of vital capacity and complementary and reserve air have been calculated in ml instead of percentages of their volumes, on account that no correlation has been observed between variability and volume magnitude of the



*Plate XV.*

Figs. 1 and 2. R 657. (See Pl. XIV, fig. 3.) Fig. 1 shows an intraretinal, chronic abscess with central fungal focus. Stain: Haem. v. Gieson. Microphotograph. Magnification, 31 diameters. Fig. 2 shows a detail picture of a border portion of the fungal focus. The latter, which consists chiefly of elongated cells, is surrounded by a hyaline border (a). Stain: Haem. v. Gieson. Microphotograph. Magnification, 280 diameters.



Fig. 1.

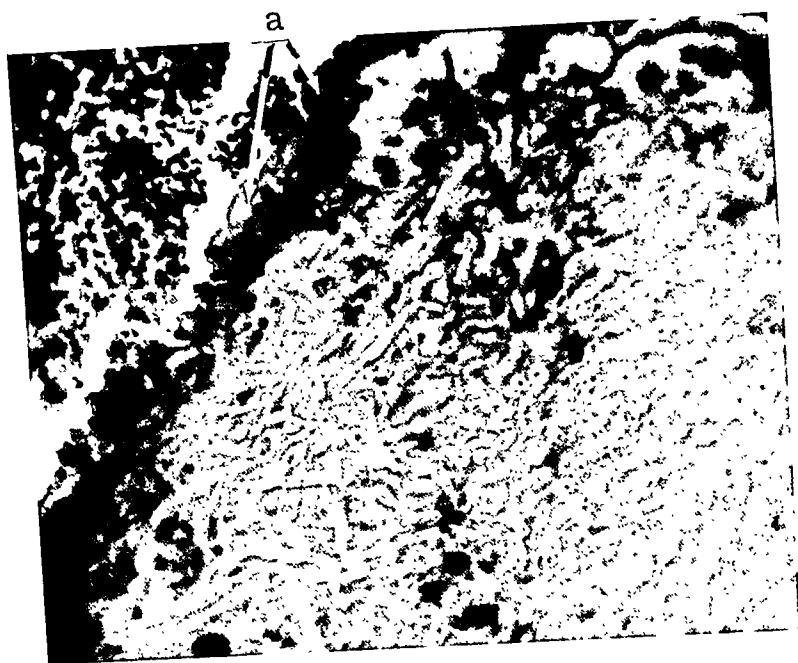


Fig. 2.

*Plate XVI.*

Fig. 1. R 527. Inoculated intravenously twice with glycerin bouillon culture of fungus strain II, and killed after about 6 months. This fig. shows a tuberculoid granuloma in the liver. At the upper border of the granuloma is seen a Langhans' giant cell. (In the lungs and spleen were found the same changes with a moderate quantity of acid-fast rods of Koch's type, as shown in Pl. VIII, figs. 1—3.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 200 diameters.

Fig. 2. R 575. Inoculated intravenously with a suspension in NaCl-solution from the spleen of R 527. The animal died after 6½ months in an extremely cachectic condition, and displayed changes with acid-fast rods in lungs, liver, spleen, kidneys, intestine, and skeleton, some of them reminiscent of tuberculosis. This photograph shows in the caecum (a) and ileum light foci scarcely the size of a pin's head, and at the top of the photograph a part of the left kidney with cicatrose pits on the surface.

Figs. 3 and 4. These photographs show respectively in the regions left elbow and left knee-joint large, nodose, caseous abscesses, issuing from osteomyelitic foci. (Acid-fast rods from a smear from the focus in the left knee are seen in Pl. VIII, fig. 5.)

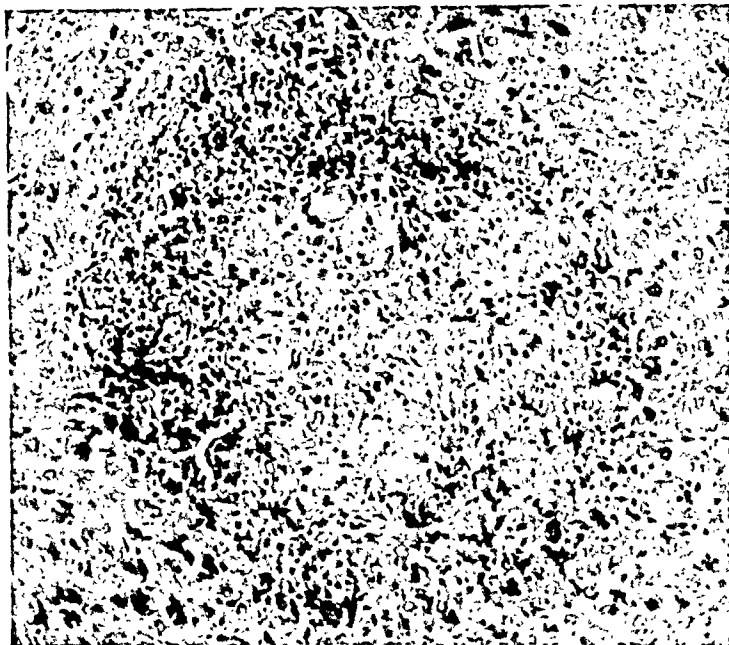


Fig. 1.

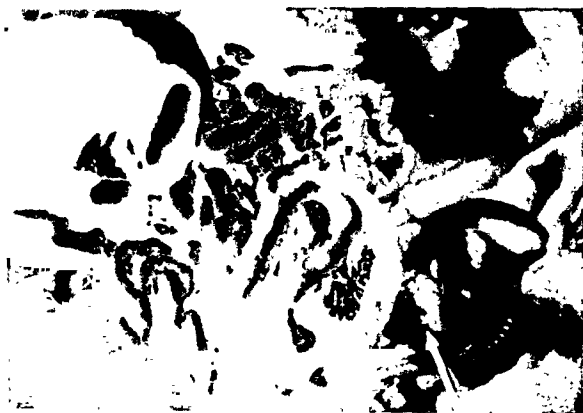


Fig. 2.



Fig. 3.



Fig. 4.

*Plate XVII.*

Fig. 1. R 575. Miliary, tuberculoid granuloma in the cortex of a kidney. Stain: Haem. v. Gieson. Microphotograph. Magnification, 75 diameters.

Fig. 2. R 575. Tubercle-like granulomata (a) with central caseous necrosis in the fibrous capsule of a larger, partly calcified, caseous abscess (b) in the fossa temporalis.

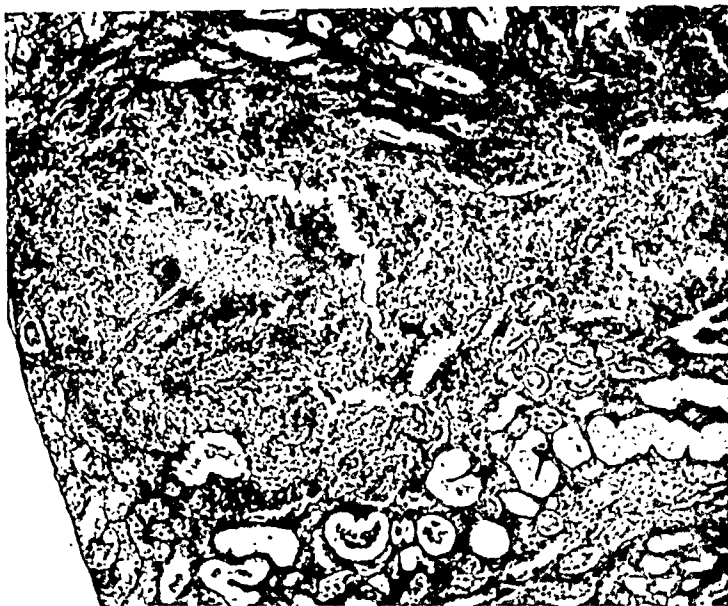


Fig. 1.



Fig. 2.

b

*Plate XVIII.*

Fig. 1 a. R 584. Inoculated intravenously with a suspension in NaCl-solution from the spleen of R 527. The animal was killed after 3 months. This fig. shows the spleen with solitary, whitish nodules glinting through the surface. (Microscopically the nodules were found to consist of epithelioid cell granulomata with numerous rudimentary giant cells, and in one and another granuloma commencing to turn into a caseous necrosis with rather plenty of acid-fast rods.) Photograph slightly reduced. At the autopsy the spleen measured  $48 \times 13$  mm.

Fig. 1 b. R 689. Inoculated intravenously with a suspension in NaCl-solution of pus from the osteomyelitic focus in the left knee-joint of R 575. This fig. shows the excessively enlarged spleen pervaded by numerous miliary foci. (Pl. XIX shows the histological picture.) Photograph in natural size.

Fig. 2. R 690. Inoculated with the same emulsion as R 689. On the left side of the picture is seen a part of the liver with numerous sub-miliary, white foci. On the right of the picture is observed a bit of one lung with numerous white foci the size of up to a pea. Photograph in natural size. (Microscopic pictures in Pl. XX and Pl. XXI, fig. 1.)

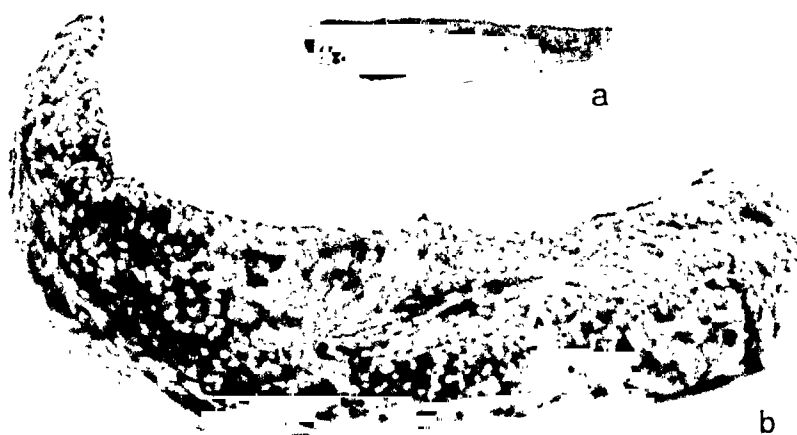


Fig. 1.

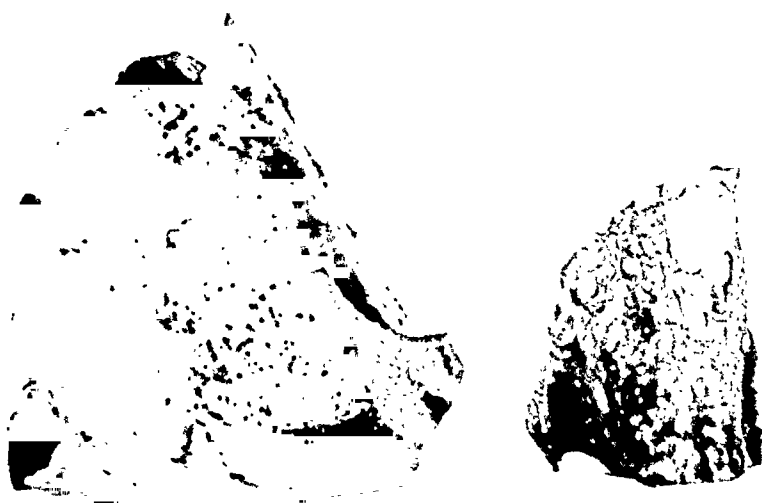


Fig. 2.



*Plate XX.*

Figs. 1 and 2. R 690. Fig. 1 shows a total view of a part of the lung (Pl. XVIII, fig. 2) with three miliary, caseous, tuberculoid foci, one of which is located subpleurally. The central focus is seen to have developed from the wall of an artery (a). Stain: Haem. v. Gieson. Microphotograph. Magnification, 20 diameters. Fig. 2. Detail view from border portion of one of the said foci in fig. 1. a) caseous necrosis, b) epithelioid cell granulomatous tissue, c) Langhans' giant cell. (In the foci a moderate quantity of acid-fast rods.) Microphotograph. Magnification, 180 diameters.

E. Hollström.



Fig. 1.

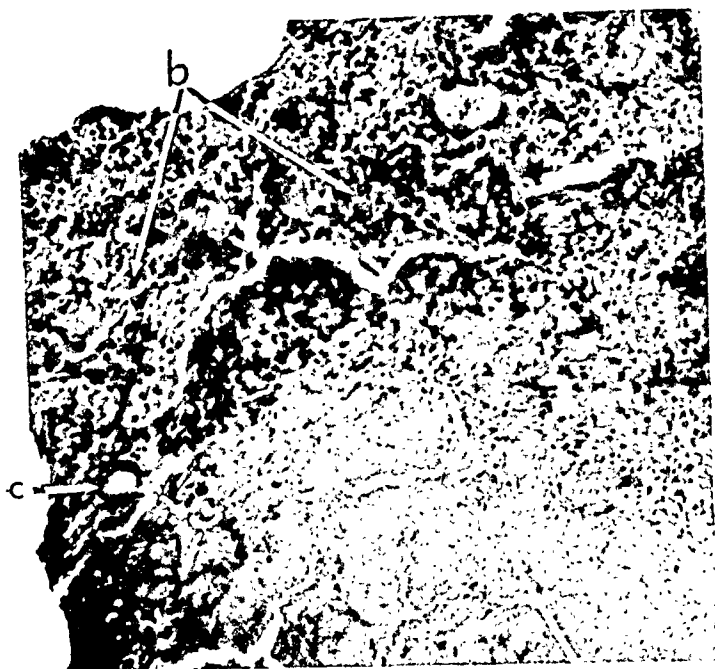


Fig. 2.

*Plate XXII.*

(Figs. 1—9. Photographs of cultures in natural size.)

Figs. 1—3. These figs. show the growth of fungus strain II on respectively ordinary agar (fig. 1) and maltose agar (figs. 2 and 3) after 10 days, 5 days at a temperature of 37° C and 5 days in living-room temperature.

Fig. 4. Culture on Löwenstein-medium after 53 days at 37° C of fungus strain III, after animal passage. (This culture contained fungus elements and short, acid-fast rods, as Pl. VI, fig. 2, shows.)

Fig. 5. Culture of acid-fast bacilli from the spleen of R 527 (Pl. VIII, figs. 2 and 3) on Löwenstein-medium after 30 days at 37° C, third generation.

Fig. 6. Culture of acid-fast bacilli from the spleen of R 584 (Pl. XVIII, fig. 1) on Löwenstein-medium after 30 days at 37° C.

Fig. 7. Culture of acid-fast bacilli from an osteomyelitic focus in the left knee-joint of R 575 (Pl. VIII, fig. 5, and Pl. XVI, fig. 4) on Löwenstein-medium after 30 days at 37° C, third generation.

Fig. 8. Culture of acid-fast bacilli from the spleen of R 689 (Pl. VIII, fig. 8, Pl. XVIII, fig. 1, and Pl. XIX) on Löwenstein-medium after 30 days at 37° C.

Fig. 9. Culture of acid-fast bacilli from a subcutaneous abscess of G-p 383 on Löwenstein-medium after 26 days at 37° C. This animal was inoculated subcutaneously in the right groin with a suspension in NaCl-solution of pus from an osteomyelitic focus of R 575.



Fig. 1.

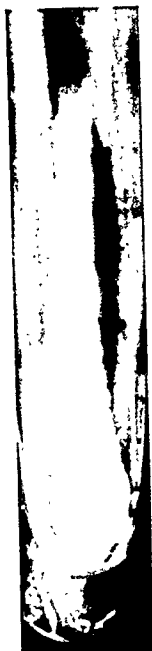


Fig. 2.

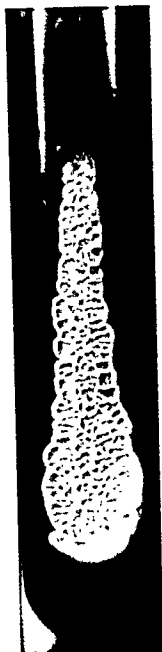


Fig. 3.



Fig. 4.



Fig. 5.

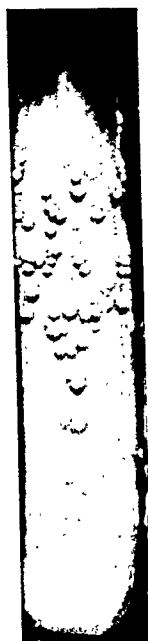


Fig. 6.



Fig. 7.



Fig. 8.



Fig. 9.



# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CXLV

1945

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## THE INFLUENCE OF HEART GLUCOSIDES, THEOPHYLLINE AND ANALEPTICS ON THE CARDIAC OUTPUT IN CONGESTIVE HEART FAILURE

WITH REMARKS ON THE ACETYLENE METHODS  
FOR THE DETERMINATION OF THE ARTERIO-  
VENOUS OXYGEN DIFFERENCE

BY

*S. BERSÉUS*

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P. A. NORSTEDT & SÖNER, STOCKHOLM 1943

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SUPPLEMENTUM CXLV

FROM THE PHARMACOLOGICAL DEPARTMENT OF THE CAROLINE  
INSTITUTE AND THE MEDICAL CLINIC OF THE ROYAL  
SERAFIMER HOSPITAL, STOCKHOLM.

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THE INFLUENCE OF HEART  
GLUCOSIDES, THEOPHYLLINE AND  
ANALEPTICS ON THE CARDIAC  
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*S. B E R S É U S*



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STOCKHOLM 1943

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## Preface.

The theoretical and technical part of the work in this investigation has been carried out at the Pharmacological Laboratory of the Caroline Institute. I take this opportunity to express my deep gratitude to Professor G. LILJESTRAND, Chief of the Laboratory. His vast experience in the physiology of respiration and circulation has been of invaluable help to me.

I also wish to convey my sincerest thanks to Professor A. KRISTENSON, Chief Physician of the Medical Clinic of the Royal Serafimer Hospital, for the great interest he has always taken in my investigations, and for having so kindly placed at my disposal the cardiac patients of the Clinic.

I am deeply indebted to Docent G. NYLIN, temporary Professor and Chief of the Medical Clinic when these investigations were commenced, as he did everything to facilitate my work and has always followed it with keen interest. I have also had the opportunity of making some determinations in his department at the Sabbatsberg Hospital, for which I also wish to express my thanks.

To Professor J. TILLGREN, who was also temporary Professor and Chief of the Clinic, I desire to express my best thanks for the kind interest he took in my work and for the favourable conditions under which I was able to continue my investigations.

I am extremely grateful to Dr. L. GOLDBERG for many valuable discussions, especially in connection with the statistical treatment of the material.

I also wish to thank my colleagues at the Medical Clinic for their kind help in selecting cases.

The technical staff of the Pharmacological Laboratory have given me skilful and careful assistance, for which I am much obliged.

Finally, I should like to express my great appreciation to Miss DOROTHY FERRIS for the care and interest she has taken with the translation.

The investigation has been supported by grants from the Swedish Society for Medical Research, from the Foundation »Therese och Johan Anderssons Minne» and from the »Lindal Fond» of the Royal Swedish Academy of Sciences.

Stockholm, April 1943.

*S. Berséus.*

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## Introduction.

The cardiac output, the amount of blood every ventricle of the heart expels, usually expressed in liter per minute, is naturally one of the factors in the physiology of circulation that has aroused most interest. A number of more or less practicable methods to determine the cardiac output have been worked out. GROLLMAN (1932) in his monography and HÖHWÜ CHRISTENSEN (1937) pass an opinion in detail on these. Broadly speaking the methods are of two kinds, namely, those in which the stroke volume is primarily determined physically, and methods by means of gas analysis.

### Physical Methods for Determining the Stroke Volume.

Among the former only LILJESTRAND's and ZANDER's (1928) blood pressure method and the sphygmographic method, worked out by BROEMSER and RANKE (1930) are of any great interest. LILJESTRAND's and ZANDER's so-called reduced amplitude-frequency product has been proved roughly to follow some changes in the cardiac output, but has failed particularly when circulation changes have been brought about by other causes than increased oxygen consumption (NEUKIRCH, 1938). The method is simple to carry out and can show the direction of the cardiac output changes, but does not give quantitative information in all circumstances.

BROEMSER's and RANKE's sphygmographic method has been largely adopted in German investigations. Its great advantages are that it does not require any co-operation on the part of the subject and that it allows of a continuous recording of the stroke volume. APERIA (1940, 1941, 1942) thoroughly examined the theoretical conditions of the method and found that the formulae hitherto fixed according to BROEMSER and RANKE, WETTERER 1940,

WETZLER and BÖGER 1936, are inadequately founded, especially in cases of tachycardia, hypertonia and in advanced age they are unsatisfactory with errors sometimes as great as 300 %. APERIA provided another formula which gives a better result. Obviously the method cannot yet be considered sufficiently worked out. The clinical results achieved with BROEMSER's and RANKE's method must be judged with great scepticism and will not be discussed again in the following.

## Methods by Means of Gas Analysis.

1. *Methods based on FICK's principle.* According to FICK (1870), oxygen consumption or carbon dioxide elimination is determined, as well as the difference between the oxygen or the carbon dioxide contents of arterial and mixed venous blood. The cardiac output is then obtained by dividing oxygen consumption or carbon dioxide elimination by the arterio-venous difference. The arterio-venous oxygen difference is of great interest in the physiology of circulation, and this is the factor that is fixed in most practicable methods for determining the cardiac output; most of the difficulties are to be found when it comes to establishing this difference. A number of direct, bloody and indirect methods have been worked out according to FICK's principle, but have only been found suitable for practical use to a limited extent. They have nevertheless been of value for the purpose of controlling other methods.

2. *Methods based on BORNSTEIN's principle.* According to BORNSTEIN (1910), an indifferent gas is used, its absorption coefficient being known. After determining the simultaneous absorption of oxygen and the indifferent gas by means of some special respiration procedure, it is possible to calculate the cardiac output. The first really practicable method to determine this function was KROGH's and LINDHARD's (1912) nitrous oxide method. This also required the determination of residual air, the time of experiment and expired volumes. These factors were eliminated by LUNDSGAARD (1915), who calculated "the stream equivalent", i. e. the quantity of blood passing through the lungs pr. 100 cc absorbed oxygen. He did not apply this consistently, however, but only in some cases, when the determination of the factors in question caused difficulties. LINDHARD (1922) introduced

the calculation of the quantity of oxygen absorbed by 1 liter of blood when passing through the lungs i. e. the arterio-venous oxygen difference, and was able to do away entirely with the determination of residual air, time of experiment and expired volumes. The oxygen consumption was determined in a separate experiment, and the cardiac output was obtained by the division of oxygen consumption by the arterio-venous difference. This principle means a considerable simplification and has also been applied in subsequent acetylene methods.

The acetylene method worked out by GROLLMAN (1929) largely facilitated the technique both for the subject and the experimenter. By means of this method numerous examinations of the cardiac output have been carried out on healthy subjects under various conditions, as well as under the influence of various drugs. It has also been possible to make use of it in clinical examinations. When adapted to pathological cases, it has been difficult to bring about the theoretical conditions for the method, this being particularly so in cardiac insufficiency. There are conflicting results to be found in literature. Generally speaking there seems to be little clarity as to the influence of drugs on the cardiac output in the case of heart insufficiency.

The purpose of this work has been to study more closely the effect on the cardiac output in congestive heart failure, firstly of the cardiac glucosides, digitalis and strophanthin, secondly of a purine derivate theophylline diaethanolamine, thirdly of the usual analeptics, metrazol (cardiazol) and nicethamide (coramin).

In experiments during rest it is reasonable not to expect any effect from these drugs on the cardiac output in cases without heart insufficiency, as then the heart works optimally and with a great reserve. When there is insufficiency, on the other hand, it may be supposed that a favourable action on the heart causes an increase in the cardiac output.

When this work was planned, it was intended to make use of the GROLLMAN method, but this soon proved not altogether suitable. A new method, recently published by E. NIELSEN (1937), was then adopted. It has, however, been found necessary to modify this in several respects. The first part of the work gives an account of these methods and the experiences derived from them, while the latter will deal with the results of the studies of drug action.



## PART I.

### Methods.

#### Grollman's Acetylene Method.

As has already been said, GROLLMAN's acetylene method was made use of at the beginning of this investigation. A short report will now be given of this method, and the critique as well as the modifications that have been put forward in the course of latter years will be discussed. Further particulars and the theory for the calculation will be found in GROLLMAN's monography (1932) and HOHWÜ CHRISTENSEN's (1937) review.

The procedure is as follows. The subject rebreathes from a rubber bag a mixture of acetylene, air and oxygen, and the bag is completely emptied at each inspiration. The depth of each breath should be about 2.4 l. for a fully grown person, the volume of gas being reduced if necessary so that it can be inspired without undue effort. When a homogeneous mixture has been accomplished in the lung-rubber bag system, a sample is taken at the end of an expiration in an evacuated gas sampling tube, and after 2—3 respirations another sample. According to MARSHALL and GROLLMAN (1928), healthy subjects require 5—6 respirations of about 15 seconds to bring about a complete mixture. The last sample should be taken within 23 seconds, as even by that time blood containing acetylene returns to the lungs and the acetylene absorption becomes less, with the result that too high a value arises in the arterio-venous oxygen difference. The samples are analysed in an HALDANE gas analysis apparatus (a modified apparatus according to KROGH-LILJESTRAND has been used in this investigation).

The arterio-venous oxygen difference is calculated according to the following formula:

$$\frac{\left( O_2 I \times \frac{N_2 II}{N_2 I} - O_2 II \right) \times \frac{C_2 H_2 I + C_2 H_2 II}{2} \times (B - 48.1) \times 0.00974}{C_2 H_2 \times \frac{N_2 II}{N_2 I} - C_2 H_2 II} =$$

= Art.-ven. O<sub>2</sub> diff.

where (O<sub>2</sub>) I, (C<sub>2</sub>H<sub>2</sub>) I and (N<sub>2</sub>) I represent the percentual percentages of oxygen, acetylene and nitrogen in the first sample, (O<sub>2</sub>) II, (C<sub>2</sub>H<sub>2</sub>) II and (N<sub>2</sub>) II corresponding values in the second sample. B is the barometric pressure in mm of mercury. The oxygen and acetylene differences in the formula should amount to at least 1 % to prevent the analytic errors from having too great an influence on the result.

The most important sources of error in the method are

- 1) Failure to achieve homogenous mixture.
- 2) Recirculation of the blood containing acetylene to the lungs before the end of the test.
- 3) Error in analysis.

### 1) Incomplete Mixture.

If the first sample is taken before the mixing has been completed, the result will be too low a value of the arterio-venous oxygen difference (GROLLMAN, FRIEDMAN, CLARK, HARRISON, 1933, E. NIELSEN, 1937). In healthy subjects homogenous mixture will be achieved as a rule, but it will be more difficult with cardiac patients, particularly those with lung congestion. GROLLMAN, FRIEDMAN, CLARK and HARRISON introduced the three sample technique in 1933 for such cases. A three sample technique was adopted even in 1931 by HOHWÜ CHRISTENSEN to control the method when determining the cardiac output at work. According to GROLLMAN et al. three samples are taken 20, 25 and 30 seconds after the beginning of the rebreathing, and 2 values of the arterio-venous difference are calculated between the 1st and 2nd and between the 2nd and 3rd samples. Should the difference between these values be less than 10 %, the authors consider this to be a guarantee for complete mixture. The time for the experiment in such cases may be prolonged to 30 seconds or

more on account of slower circulation in these patients. GROLLMAN et al. (1933), found in hydrogen experiments ad modum LUNDSGAARD and SCHIERBECK (1923) that it took longer to achieve the mixture and they recommended that the 1st sample should not be taken until after 20 seconds. They also found that the acetylene equilibrium between alveolar air and blood is not prevented by congestive heart failure.

E. NIELSEN (1937) expressed doubt as to the possibility of achieving complete mixture on the basis of experiments in which he collected many samples in the same rebreathing period, and found that the values of the arterio-venous  $O_2$ -difference increased the whole time, even though the time of the test did not exceed 25 seconds. He also refers to SONNE's (1936) theory on the unequal function of the lung even in normal subjects. According to SONNE, this seems, moreover, to become considerably more pronounced during deep respirations. NIELSEN's experiments are not, however, completely convincing. A certain premature recirculation from short circuits, above all from the coronary circulation, cannot be avoided either, a fact that GROLLMAN emphasizes. Most authors having experience of the method consider that complete mixture can generally be achieved except in some pathological cases, which are then discovered by the three sample technique. On the other hand, several authors maintain that it is possible to obtain complete mixture much sooner (GLADSTONE, 1935; McMICHAEL, 1937; ADAMS and SANDIFORD, 1941). They consider that the hydrogen experiments carried out by GROLLMAN et al. show complete mixture later than is actually the case and refer to a work on the rebreathing procedure by GLADSTONE and DACK (1935). These authors find a progressive fall in the respiratory quotient during the rebreathing with a rise in volume during the first three breaths and a progressive fall thereafter, and emphasize the inadequacy of any mixing criteria which are based on percentage concentration of an inert gas without due consideration to the concomitant changes in total volume. As opposed to these authors NIELSEN (1937) considers that the circumstances in hydrogen experiments, in which the volume of gas is more constant, are far more favourable for complete mixture than in the acetylene experiments, in which the acetylene quantity diminishes considerably, and that mixture in hydrogen experiments does not necessarily guarantee mixture in acetylene experiments.



McMICHAEL (1937) recommends to make breathing less deep with a tidal air of only 1—1.5 litre, as is often necessary on patients. In this way the acceleration of the circulation will be less pronounced. He finds several more good checks in the three sample technique with this alteration. He recommends samples to be taken at the 4th, 6th and 7th or 8th respiration. In these circumstances it seems somewhat optimistic to expect complete mixture as soon as at the 4th respiration. ADAMS and SANDIFORD (1941) recently published a modified method by which they take multiple samples, partly of "bag gas", partly of "alveolar gas", which was made possible by a flap valve between the bag and the sampling tube connexions at the mouthpiece. In determining the arterio-venous difference from alveolar gas and bag gas samples, they think it is possible to identify the establishment of lung-bag equilibrium and the beginning of recirculation. They find that the time required to achieve lung-bag equilibrium and the time of the first appearance of recirculation are variable, but that they usually occur much earlier in the period of rebreathing than GROLLMAN indicates.

### 3) Analytical Errors.

The ordinary HALDANE gas analysis apparatus gives an accuracy of  $\pm 0.02$ — $0.03$  %. A source of error in the analysis is that the acetylene is slightly soluble in the absorption liquid for carbon dioxide. By means of an absolutely uniform analysis technique this error can be kept so constant that it has no practical influence on the magnitude of the acetylene difference and consequently not on the value of the arterio-venous oxygen difference. McMICHAEL (1937), however, has been impressed by this analytic error. He points out that a greater quantity of acetylene should be dissolved at the beginning of an analytic series instead of later, as by that time the solution has become more saturated. In order to avoid this he has worked out a method of analysis using VAN SLYKE's manometric apparatus, which also allows of the analyses of higher acetylene concentrations. Acetylene mixtures of such high percentages are used that the contents in the samples are 12—15 % and the acetylene differences, 3 à 4 % instead of about 1 % as is the case with the GROLLMAN method. This is naturally a great advantage. Very strong acetylene mixtures are, however, a disadvantage, as will be later

on discussed. This method has undoubtedly its advantages, but it complicates the work of analysis. No other authors seem to have made use of it either.

### The Author's Experiences with the Grollman Method.

As has been already said at the beginning of this work, GROLLMAN'S three sample technique has been tried on cardiac patients. The method has also been adopted in some series of experiments on normal subjects but only with two samples as a rule.

In some experiments on normal subjects 3 samples have also been taken with a view to getting the question of recirculation further elucidated. From this material the values of the arterio-venous differences have been compared in two types of experiments. In one type samples have been taken after 6, 8 and 9 respirations at about 18—24—27 seconds respectively, in the other group samples have been taken after 6—9—10 respirations at about 18—27—30 seconds respectively. The subjects have been both healthy persons as well as patients with vitium org. cordis or hypertonia without insufficiency symptoms, for whom the rebreathing procedure has not caused any difficulties. Complete mixture after 6 respirations and 18 seconds may be said to have occurred. In every experiment the arterio-venous oxygen difference has been calculated partly from the samples after 6 and 8 or 9 respirations, the 1st value, partly from samples after 6 and 9 or 10 respirations respectively, the 2nd value. The difference 2—1 in every experiment has been calculated and the differences worked out statistically. The result is to be seen in table 1.

Table 1.

	1st values O <sub>2</sub> diff. 6—8 respirations	2nd values O <sub>2</sub> diff. 6—9 respirations	Diff. 2—1
Mean	56.49 cc/l	57.06 cc/l	+ 0.58 cc/l
n = 22			± 0.951
8 subjects			
	1st values O <sub>2</sub> diff. 6—9 respirations	2nd values O <sub>2</sub> diff. 6—10 respirations	Diff. 2—1
Mean	60.27 cc/l	62.92 cc/l	+ 2.65
n = 30			± 0.588
8 subjects			

Thus it seems as if recirculation in normal subjects is of no importance up to an experiment time of about 27 seconds, on the other hand it has begun to make its appearance after about 30 seconds causing an increase in the arterio-venous difference of about 5 %.

As double determinations under standard conditions have been consequently carried out, the standard deviation for a single determination  $\delta_t$  can be calculated by dividing the standard deviation of the differences in double determinations by  $\sqrt{2}$  (DAHLBERG, 1940). The standard deviation of the differences  $\sigma_d$  has been calculated according to the usual formula.  $\sigma_t$  naturally includes both normal physiological variations as well as those due to errors in method. The result will be seen from table 2.

Table 2.

Number of double determinations in healthy subjects . . . . .	29	Difference II-I
Mean of 58 single determinations . . . . .	61.14 cc/l	Mean + 0.50 cc/l
		$\pm 1.45$
		$\sigma_d = \pm 7.82$
Standard deviation of a single determination . .	$\sigma_t = \pm 5.536$	
Coefficient of variation . . . . .	9.05 %	

In patients with congestive heart failure the three sample technique of the GROLLMAN method has been tried. In these cases the rebreathing procedure often involves great difficulties for the patients. In some cases it is quite impossible to carry it out. Samples have usually been taken after about 18—20 seconds, 25—30 seconds and 30—35 seconds. The values calculated from the 2nd and 3rd samples have had to be rejected in most cases, the acetylene difference being considerably under 1 %, this in spite of the fact that the acetylene percentage in the mixture used had been raised to 30 à 35 %. A further increase means greater discomfort for the patient. An increase to over 50 % seems to be necessary (NIELSEN 1937, ESPERSEN 1939). This does not seem to be entirely without risk, Nielsen having observed vomiting and a deterioration of decompensation symptoms. If the values that are calculated between the 1st and 2nd samples and between the 1st and 3rd are compared, an idea can be formed as to the recirculation, but no reliable control can be ascertained that complete mixture has been achieved. Table 3.

Table 3.

Grollman determinations in decompensated patients (three sample technique).<sup>1</sup>

	Value 1, calculated from 1st and 2nd samples	Value 2, calculated from 1st and 3rd samples	Difference II—I
	Art.-ven. O <sub>2</sub> diff.	Art.-ven. O <sub>2</sub> diff.	
Mean	84.20 cc/l	88.86 cc/l	+ 4.66 cc/l
n = 40			± 0.843
			$\sigma = \pm 5.329$

This significant difference will probably be regarded as being caused by recirculation, and in the majority of cases the time for the experiment should not be more than 30 seconds.

That it has been possible to obtain constant values in spite of this fact will be seen from the following series of double determinations under standard conditions, 10 determinations on 6 patients with congestive heart failure. Table 4. The mean of the values calculated between the 1st and 2nd and the 1st and 3rd samples has been taken as the values of the arterio-venous difference in each particular experiment.

Table 4.

	I values	II values	Difference II—I
Mean n = 10	91.24 cc/l	91.52 cc/l	+ 0.28 cc/l
6 subjects			± 2.223
			$\sigma_d = \pm 7.029$
Standard determination for a single determination.....			$\sigma_t = \pm 4.970$ cc/l
Coefficient of variation: 5.45 %.			

When judging this low coefficient of variation, cf. 9.05 % of the normal material, it must be remembered that this material is to a certain extent selected, owing to the fact that the method has not been able to be carried out on a number of patients or if so, it has given such obviously erroneous results that they have been rejected and no further experiments have been made. Moreover, every value in this series is the average of two in accordance with the three sample technique. A partial explanation may be that the patients with an insufficiency of circulation have considerably smaller spontaneous variations than healthy people, whose cardiac output in repose is, however, somewhat labile.

<sup>1</sup> The primary material in this table includes firstly double determinations under standard conditions, secondly some single determinations and thirdly determinations under the influence of digitalis. This is the cause of the difference in the mean values of the arterio-venous difference between table 3 and the following table 4.



As it seemed desirable in further experiments to shorten the time to about 30 seconds, the author subsequently took less between the 2nd and 3rd samples when applying the three sample technique. Two values of the arterio-venous difference were calculated by the help of the 1st and 2nd as well as the 1st and 3rd value. In order to form an idea of the possibility of complete mixture, preparatory hydrogen experiments were made. MARSHALL and GROLLMAN (1928) and GROLLMAN, FRIEDMAN, CLARK and HARRISON (1933) made similar experiments ad modum LUNDSGAARD and SCHIERBECK (1923). When making use of this technique, simultaneous samples are taken during rebreathing at the end of the expiration of alveolar and bag air respectively. When a positive difference is obtained between alveolar samples and bag samples, equilibrium is considered to be achieved in the lung-bag system. It seems more simple, and theoretically more acceptable to make use of the same technique as in the acetylene rebreathing experiment. Samples are taken at the end of some successive expirations. When equilibrium has been obtained, the hydrogen percentage will be constant, if consideration is taken to the change of volume during the rebreathing procedure by means of correction with the help of the nitrogen concentrations. When analyzing it is only necessary to determine the  $H_2$ - and  $N_2$ -concentration, and therefore  $CO_2$  and  $O_2$  can very well be absorbed all at once in the alkaline hydrosulfit solution used in the oxygen absorption. Some examples will be given.

Normal subject (medical student, 21 years of age):

	Sample 1.	2.	3.
	5th respiration 17 seconds	6th 22 seconds	7th 25 seconds
$H_2\%$ . . . . .	8.05	8.08	8.12
$N_2\%$ . . . . .	67.33	67.70	68.03
$H_2$ corr. . . . .	8.14 (corr. ad 3)		
	8.09 (corr. ad 2)		

Equilibrium has been obtained after 5 respirations of 17 seconds respectively.

O. J. ♀ 32 years of age. Vit. org. cordis incomp.

	Sample 1.	2.	3.
	5th respiration 17 seconds	6th 22 seconds	7th 25 seconds
$H_2\%$ . . . . .	8.05	8.09	8.11
$N_2\%$ . . . . .	67.34	67.71	68.05
$H_2$ corr. . . . .		8.05 (corr. ad 1)	8.03 (corr. ad 1)

in some places other forms. The number of well preserved *lymphocytes* and *lymphoblasts* is much smaller than in the normal lymphadenogram. This is explained by the increased number of Gumprecht's shadows. *Reticulo-endothelial* cells were not observed, neither were *granulocytes* seen; some erythrocytes are present.

*Chronic myeloid leucemia.* The erythrocytes when compared with a normal lymphadenogram show no differences. *The granuloblastic system:* In all fields of vision there are several or so typical myeloblasts (Fig. 12), and somewhat smaller proportion of metamyeloblasts and promyelocytes, and still smaller number of neutrophil myelocytes, metamyelocytes, stab cells and polymorphs. Only adult forms of oxyphil and basophil granulocytes were observed. The majority of the cells were well preserved, the rest however showed the cytoplasm to be more or less damaged with partial and even complete separation from the nucleus. From these separated parts the basophil fragments of cytoplasm arise. They are usually spherical, of different size, from that of normocyte to small round granules, which at times fill the entire field of vision. The nuclei deprived of cytoplasm undergo disintegration giving origin to *Gumprecht's shadows*. *Lymphocytes* are less numerous than in a normal gland and typical *lymphoblasts* only exceptionally were observed. Typical *reticular cells* appear as a rule in the same proportion as in normal lymphadenogram. Many cells in apparent transition from reticular cells to myeloblasts were observed. *Plasma cells* were scarce.

*Acute myeloid leucemia.* On examining the smears from enlarged cervical glands the following was confirmed: In the *erythroblastic system* scanty nucleated red cells and a few erythrocytes with fairly marked aniso- and poikilocytosis were seen. *Lymphocytes* and *lymphoblasts* were far less numerous than in normal lymphadenogram. In the *granuloblastic system* there were many typical myeloblasts (Fig. 13), often showing signs of degeneration and disintegration with development of Gumprecht's shadows. There were no more differentiated forms — from metamyeloblasts to adult granulocytes. A few cells with the characteristics of *reticular cells* were seen; their structure is on the whole similar to that of myeloblasts, but they are a little larger.

Our material, though it is very modest, allows us to draw the following conclusions concerning the value of the lymphadenogram in diagnosis of leucemia: The lymphadenogram in the course of chronic lymphatic leucemia, in leucemic as well as in aleucemic

by the author and used in a somewhat modified form for most of the studies of drug action on cardiac output in this investigation.

The following is a brief description of the principles of NIELSEN's method. The subject breathes under normal respiration from a DOUGLAS bag through a valved apparatus a constant gas mixture of 21 % oxygen and about 7 % acetylene as a test mixture. When a state of equilibrium in the lungs has been formed, a sample is taken of the expiration air at the end of one or several expirations. Thanks to the normal respiration, an increase in circulation resulting from hyper ventilation as in the rebreathing procedure is avoided. This explains why the time for the experiment can be prolonged to about 50 seconds in normal subjects before the recirculation begins to be upsetting.

The arterio-venous oxygen difference is then calculated from samples of the inspiration as well as the expiration air according to LINDHARD'S (1923) formula. The percentage in the sample taken at the end of a normal expiration is selected as the value of the alveolar mean concentration for the acetylene.

In order to bring about equilibrium within a reasonable time, the subject must first inhale from a small bag a suitable quantity of a considerably stronger acetylene mixture, the primary mixture, after which the test mixture is turned on. Suitable quantities of the primary mixture of an appropriate strength are tested in a preparatory experiment with corresponding gas mixtures containing hydrogen instead of acetylene. When equilibrium takes place the hydrogen concentration will be the same in the inspiration and expiration air, if correction is made for the change of volume with the help of the nitrogen concentrations.

NIELSEN keeps the primary mixture constant with 20 % acetylene in his first experiments and varies the test mixture. It is more practical however, to reverse the order.

The oxygen percentage in all gas mixtures should be kept at 21 % as in normal air, thus facilitating the establishment of equilibrium, and in addition to this the oxygen tension attained in the alveoli during the test will be the usual one for the subject. With the GROLLMAN method the oxygen tension varies during the test, and it is a mere chance if the mean concentration is the usual one for the subject. This is a source of error worthy of note in cases of reduced arterial oxygen saturation, which was pointed out by HÖHWÜ-CHRISTENSEN and H. E. NIELSEN (1936).

NIELSEN considers it advisable to take a continuous sample for 35—45 seconds after the subject has begun to breathe acetylene, as he has found agreement between the value of the arterio-venous difference calculated with this sample and the mean of the values calculated of several momentary samples taken in the same space of time.

In a series of experiments on 5 different normal subjects, where the open system according to NIELSEN and the GROLLMAN method have been adopted simultaneously, NIELSEN finds good agreement between the two different methods, as well as less spreading with the open system.

NIELSEN has had difficulties with cardiac patients when making use of strong primary mixtures. Consequently he has used an acetylene concentration of only 14 % in these cases. In order to bring about equilibrium in such circumstances, he has worked out complicated processes with hyperventilation and pauses in respiration, which have largely eliminated the advantages of the method. As only technique with normal respiration has been made use of in this work, these modifications will not be discussed further.

ESPERSEN (1939, 1940, 1941) used a somewhat modified form of NIELSEN's method when examining cardiac patients. He finds no particular difficulties when using acetylene concentrations up to 30—40 % in the primary mixture and has given up the hyperventilation modifications. He makes use of a complicated technique, for besides taking samples at the end of a normal expiration, he also collects the expiration air in a rubber bag during some respirations. He calculates the arterio-venous oxygen difference partly with the help of samples of the mixed expiration air when the acetylene percentage at the end of normal expiration is taken as a mean concentration, and partly only with the sample at the end of normal expiration. In some experiments the acetylene percentage at the end of a deep expiration has also been used as the value of the mean concentration. Finally by determining the time for the collection of the expiration air and its volume, he has calculated the cardiac output direct. This technique is unnecessarily complicated, nor does it seem to possess any particular advantages, and introduces, moreover, further possibilities of error, e. g. in the determination of time and volume during so short a test as this concerns, and in the manipulation of the rubber bag used when collecting the expiration air.

## The Author's Modifications of the Nielsen Method.

The apparatus is in agreement with that originally used by NIELSEN and will be seen in fig. 1. DB is a DOUGLAS bag of about 100 liter capacity and contains the test mixture with about 8 % acetylene and 21 % oxygen. RB is a smaller rubber bag containing a suitable primary mixture, 25–30 % acetylene and 21 % oxygen.

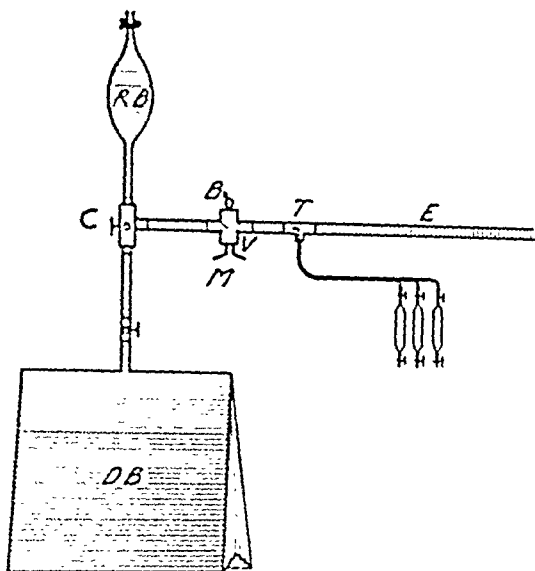


Fig. 1. The apparatus for determining the arterio-venous oxygen difference according to the Nielsen method.

The gas mixtures were prepared in the following manner. The primary mixtures, only small quantities of which are required, were prepared in a graduated mixing spirometer of 10 liter capacity, the liquid of which was made up of thin oil. First of all a suitable quantity of acetylene was measured out, usually from 2.8–3.3 liter, oxygen corresponding to  $\frac{1}{2}$  of this quantity and air ad 9 liter. The analysis of the primary mixture is unnecessary, sufficiently constant gas mixture being obtained by always carrying on in exactly the same manner. The primary mixtures are suitably kept in small rubber bags of 10–20 liter. For the test mixture, first 7 liter acetylene were measured in the mixing spirometer and 2.3 liter oxygen were then added, after which these quantities were transferred to the well emptied DOUGLAS bag. Then 60 liter air was measured in a big spirometer, and was also transferred to the DOUGLAS bag. The gases were then thoroughly mixed by beating and knocking the bag about. The gas mixtures were freshly prepared every day that experiments were made, as it was found that

by keeping the test mixture in the bag for 24 hours the acetylene percentage sank from c. 8 % to c. 7 %. In order to simplify matters the dead space in the mixing spirometer and the bag was always filled with air when the gas mixtures were being prepared.

The bags are connected by a four-way cock C with a valved apparatus V (of the Lovén type) with a mouthpiece M, so that the inspiration side of the valve can be connected either to the free air, to RB or to DB. In a T tube (T) there is placed a capillary glass tube of c. 0.8 mm inner diameter, through which the samples are taken. The capillary glass tube is connected with 3 evacuated sample tubes by means of a capillary rubber tube. This latter tube is always kept closed by 2 clips, one at each end, except when the samples are being taken. As these vary but little in their composition, the dead space of the tube is filled with gas of almost the same composition as the samples; the effect of the dead space of the tube is thus practically eliminated. A Douglas bag can be attached to the rubber tube E by a three-way cock to collect the expiration air during the breathing. The determination of the oxygen consumption is most suitably carried out according to the DOUGLAS' (1911) bag method, which only entails slight breathing resistance. At first a Krogh's spirometer was used instead, but its resistance caused discomfort to some dyspnoic patients.

The valve V is an ordinary Lovén valve with membranes of gold beater's skin. The modern types with rubber membranes offer very little breathing resistance, it is true, but they are unsuitable, as they do not remain air-tight at the slight additional pressure in the DOUGLAS bag (c. 2 mm  $H_2O$ ). There is a small thin rubber bladder B on the valve connected with the lumen, by means of which the different phases in the breathing can be easily followed.

The determination of the arterio-venous oxygen difference is carried out in the following manner. The subject, after being connected with the apparatus and provided with nose clips, breathes with normal respiration from outside air through C and into the open air through the rubber tube E. The dead spaces between the bags and the four-way cock were previously filled with the gas mixtures. During an expiration the subject is first connected with RB which contains the stronger primary mixture. A control clock is started at the beginning of the first acetylene inspiration. After a suitable number of respirations, DB is connected with the test mixture also during an expiration, and the respiration

proceeds. After about 35 seconds a sample is taken of the expiration air at the close of an expiration, another 2 samples being taken after about 40—45 and 45—50 seconds respectively. The sampling can be continued, which will most often be necessary, after the beginning of the following inspiration. A sample is also taken of the test mixture. Three values of the arterio-venous oxygen difference can then be calculated according to LINDHARD'S formula (cf. formula of GROLLMAN'S method p. 11):

$$\frac{O_2 \text{ insp.} \times \frac{N_2 \text{ exp.}}{N_2 \text{ insp.}} - O_2 \text{ exp.}}{C_2H_2 \text{ insp.} \times \frac{N_2 \text{ exp.}}{N_2 \text{ insp.}} - C_2H_2 \text{ exp.}} \times C_2H_2 \text{ exp.} \times (B-48.1) \times 0.00974. = \text{Art.-ven. } O_2 \text{ diff.}$$

At first preparatory hydrogen tests were used in exactly the same manner in order to determine the suitable strength and

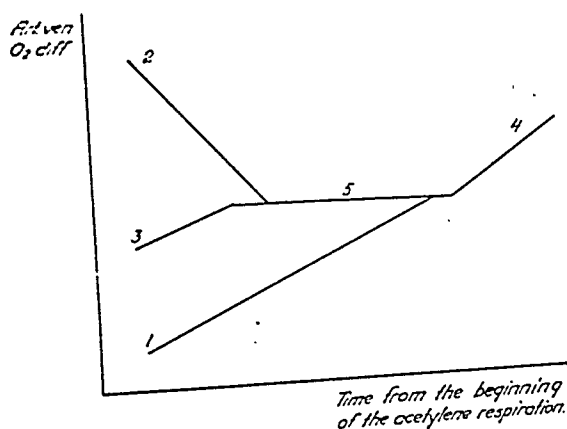


Fig. 2. The changes in the value of the arterio-venous oxygen difference in different mixing conditions. (According to Nielsen, 1937.)

number of respirations of the primary mixture, as recommended by NIELSEN. Fig. 2 (according to NIELSEN) illustrates schematically how the value of the arterio-venous difference changes with different mixing conditions. The plateau formed part 5 of the curve gives the correct value of the arterio-venous difference. In part 4 the recirculation has begun to make its appearance, 1 indicates too little primary mixture, making equilibrium very late, or else preventing it altogether in time for the test, 2 indicates too great a quantity of primary mixture, in which case the acetylene absorption facilitates the beginning of equilibrium. 3 indicates a fairly correctly chosen mixture for the rapid achievement of equilibrium. It was soon found that in consecutive

determinations on the same subject in several tests there were often lower values than were expected in the arterio-venous difference. There was then a clearly rising tendency in the values calculated in the various samples, and the acetylene concentration also showed a rising tendency, in spite of close agreement in the carbon dioxide concentration. The quantity of primary mixture had apparently been too small to achieve equilibrium.

It seems fairly natural that the quantity of primary mixture giving equilibrium in the hydrogen easily becomes too small in the acetylene experiment, for in this case a considerable acetylene absorption takes place. ESPERSEN also states that in his hydrogen experiments he has endeavoured to obtain a somewhat higher hydrogen percentage in the lungs than the concentration corrected in the test mixture. Taking into consideration, moreover, that the strong mixture is somewhat unpleasant and that the subject is inclined to counteract it by respiring less deeply, it further emphasizes the uncertainty of presuming equilibrium in acetylene experiments, simply on account of its having been achieved in an earlier corresponding hydrogen experiment, as both authors, NIELSEN and ESPERSEN, having made use of NIELSEN's method, have done.

Based on these view points the author has considered it necessary to take several samples in every experiment in order to be convinced that equilibrium has really been achieved. As the conditions illustrated in fig. 2 concerning the arterio-venous  $O_2$  difference also apply to the acetylene concentration, it is possible to judge the experiment from the equilibrium view point from the analytical figures direct. When equilibrium has been established constant values of the acetylene percentage in the samples are all but obtained, provided that they have been taken during the same phase of the expirations. Should this not be the case, there will be a change in the concentration of all the gases, chiefly due to the more or less complete washing out of the dead space, as well as variations in time for the exchange of gases. Then the acetylene and the oxygen concentrations are changed to the same direction, the carbon dioxide taking an opposite one, this making it possible to judge whether equilibrium exists. If so, the relation between the oxygen and the acetylene absorption in the formula is not changed, and variations in the value of the arterio-venous difference will be largely adjusted, if the same value of the acetylene mean concentration is inserted in the for-



mula. In the preparatory experiments, too, acetylene has consequently been used, and only carbon dioxide and acetylene analyses have been carried out. This will be sufficient when judging the experiment from the equilibrium point of view, and thus save the time-wasting process of oxygen analysis. In this manner more important errors due to failing equilibrium can be excluded. A couple of examples will illustrate the procedure.

The analytical values in a determination on a decompensated patient under standard conditions were as follows:

Test mixture <sup>1</sup>	CO <sub>2</sub> 0.25 %	C <sub>2</sub> H <sub>2</sub> 8.45 %	O <sub>2</sub> 20.81 %	Art.ven. O <sub>2</sub> diff.
Sample 1 . . . . .	3.59	6.30	16.89	84.5 cc/l
" 2 . . . . .	3.55	6.66	16.97	104.9
" 3 . . . . .	3.66	6.61	16.87	101.9
				103.4

In sample 1 equilibrium was evidently not achieved, the acetylene percentage still clearly showing a rising tendency. In samples 2 and 3, however, equilibrium probably exists. The values of the arterio-venous oxygen difference, moreover, are in agreement. As has been previously mentioned (page 24), this is calculated from the sample of the inspiration air as well as one of the expiration. Three values can thus be calculated, if 3 samples are taken of the expiration air. In a sample taken 30 mins. earlier on the same patient the figures were.

	CO <sub>2</sub>	C <sub>2</sub> H <sub>2</sub>	O <sub>2</sub>	Art.ven. O <sub>2</sub> diff.
Sample 1 . . . . .	3.40 %	6.30 %	17.12 %	80.4
" 2 . . . . .	3.72	6.52	16.57	102.9
" 3 . . . . .	3.59	6.67	16.75	108.3
				105.6 cc/l

Equilibrium seems quite probable in samples 2 and 3, in any case there can be no question of any considerable error. Compare also the above value of 103.4.

A determination of a patient with V.O.C. without signs of insufficiency:

	CO <sub>2</sub>	C <sub>2</sub> H <sub>2</sub>	O <sub>2</sub>	Arte.vn. O <sub>2</sub> diff.
Sample 1 . . . . .	4.36 %	5.30 %	16.22 %	57.5 cc/l
" 2 . . . . .	4.90	5.17	16.32	57.4
" 3 . . . . .	4.98	5.22	16.38	58.0
				57.6 cc/l

<sup>1</sup> The test mixtures are not indicated in the following examples, their being of no special interest from the equilibrium standpoint.

In this experiment equilibrium was achieved in all the samples. In another determination in the same subject 30 mins. later and still under standard conditions the result was as follows:

	CO <sub>2</sub>	C <sub>2</sub> H <sub>2</sub>	O <sub>2</sub>	Art. ven. O <sub>2</sub> diff.
Sample 1 . . . . .	5.01 %	4.15 %	15.11 %	44.6 cc/l
" 2 . . . . .	5.32	4.20	14.81	47.4
" 3 . . . . .	5.35	4.38	14.89	50.9

Equilibrium was evidently not achieved in samples 1 and 2 although it cannot be excluded in the last one. Compare value 57.6 cc in previous determination.

In most cases it has not been possible to bring about equilibrium in the very first sample. Attempts to achieve it by a further increase in the dose of primary mixture have often resulted in obvious overdosing, with falling acetylene concentrations, as well as too high but falling values of the arterio-venous oxygen difference. It seems advisable not to expect equilibrium until after about 40 seconds in patients with congestive heart failure. Consequently in this investigation the average of the values calculated with the help of the 2nd and 3rd samples, taken at 40—45 and 45—50 seconds respectively, has been used as the value of the arterio-venous oxygen difference obtained in the respective determinations. The 3rd sample has occasionally been extended to 55 seconds at most. During the latter part of the investigation it was often only the last two samples that were analyzed, although three were always taken.

By means of this technique even values and good agreement have been obtained in double determinations. The statistical treatment of all double determinations under standard conditions gave the following result. (Table 5.)

Table 5.

*Double determinations of the arterio-venous oxygen difference of decompensated patients under standard conditions.*

	Values I	Values II	Difference II—I
Mean n = 46 . . . . .	97.40 cc/l	98.90 cc/l	+ 1.50 ± 1.169 cc/l
Mean of I and II . . . . .	98.15 cc/l		$\sigma_d = \pm 7.925$
Standard deviation of a single determination . . . . .			$\sigma_t = \frac{\sigma_d}{\sqrt{2}} = \pm 5.604 \text{ cc/l}$
Variation coefficient: 5.71 %.			

It is natural that judging the equilibrium in the manner above described may often give somewhat uncertain results. A closer examination of all the determinations according to NIELSEN's method in decompensated cardiac patients included in this work, and the comparison between the values calculated with 2nd (40 seconds) and 3rd samples (50 seconds) give the following result. (Table 6.)

Table 6.

*Comparison between the values of the arterio-venous difference calculated from the 2nd sample (about 40 seconds), and the 3rd sample (about 50 seconds).*

	2nd sample	3rd sample	Difference 2-3
Mean. . . . .	95.58 cc/l	95.23 cc/l	+ 0.35 $\pm$ 0.328
n = 197			$\sigma_d = \pm 4.609$
Mean of 2 and 3 . . . . .	95.41		
Standard deviation of a single sample determination . . .	$\sigma_m = \pm 3.259$ cc/l		
Variation coefficient: 3.42 %.			

From table 6 it will be seen that no difference has been proved between the values calculated with the help of 2nd and 3rd samples respectively. Thus no influence of failing equilibrium and recirculation can be traced in the material.

It seems probable that the above standard deviation  $\sigma_m$  must mainly be attributed to the method, as it is hardly likely that changes could take place in the subject in so short a time. The variation in table 5, on the other hand, includes both the variation of the method and the physiological variation of the subject. Under these assumptions this last variation may be calculated according to the formula:  $\sigma_i = \sqrt{\sigma_t^2 - \sigma_m^2}$

$$\sigma_i = \pm 4.559 \text{ cc/l.}$$

Variation coefficient 4.65 %.

### Sources of Error.

The recirculation and the failing equilibrium have already been discussed. The analytical errors are of very little importance, as both the acetylene and the oxygen differences usually amount to several percentages. The acetylene absorption in the alkaline solution used for carbon dioxide absorption makes the acetylene values somewhat too low. When analyzing the test mixtures with

c. 8. % acetylene, the values of the  $\text{CO}_2$  percentage have been round 0.25 %. The acetylene absorbed will be estimated at 0.20 %. The acetylene percentage in the samples of the expiration air can be presumed to be c. 0.10 % too low. These errors counteract each other in the equation and have practically no influence on the value of the arterio-venous oxygen difference.

The effect of the dead space forms an important source of error. The acetylene percentage in a sample taken at the end of the expiration has been chosen as the value of the alveolar mean concentration when calculating the arterio-venous oxygen difference. To justify this there must be so great a depth of respiration that the dead space is washed through. In the event of insufficient depth of respiration, the acetylene percentage in the samples at the end of the expiration will be higher than the real alveolar mean concentration. The value of the arterio-venous oxygen difference will then be too high. The relation between the oxygen and the acetylene absorptions varies, on the other hand, only little in the course of a normal expiration (NIELSEN 1937, ESPERSEN 1939). The dead space in the apparatus used from the mouth-piece to the place where the sample was taken is 105 cc. The dead space in the subjects themselves certainly varies rather considerably, but may probably be estimated at 90—120 cc (SIEBECK 1911, KROGH and LINDHARD 1913, 1917). If a volume 3 times as great as that of the dead space (this will be somewhat exaggerated) is required to completely wash it out, then at least 600 cc depth of respiration would be required to obtain the correct values. The error will be all but eliminated at about 500 cc depth of respiration. ESPERSEN, in whose apparatus the dead space "from the valve to the place where the sample was taken" was 80 cc, considers that the error will seldom be greater than 10 %, and that certain cardiac patients in particular would have a tendency for too superficial breathing. The author's impression is that decompensated patients usually have sufficiently great depth of respiration on account of their dyspnea, while subjects without cardiac insufficiency often have more superficial breathing. Some subjects showed a tendency to more superficial breathing than normally during the actual acetylene respiration. When making parallel experiments on normal subjects with the GROLLMAN and the NIELSEN techniques, the author obtained good agreement at first between the results of the two methods, just as NIELSEN had done, but later on, however, the NIELSEN values

were often higher than the GROLLMAN, sometimes going up 15–20 %. This is obviously an uncertainty of considerable moment if it is a question of fixing the absolute value of the arterio-venous difference, and also of comparing the values of the various subjects. On the other hand, a possible error seems to be constant in the same subject at one and the same time, as will be seen (table 5) from the constant values under standard conditions in patients with congestive heart failure. Presuming that the depth of respiration does not change to any great extent, variations in the cardiac output can therefore be established at one and the same time, which is the object of this work. On the other hand, it seems to be more difficult with this form of method to study the variations in the cardiac output during the course of and after the conclusion of a decompensation.

With the object of eliminating the influence of the dead space and rendering the method generally serviceable, the author has tried various modifications. Firstly the patient was asked to make a deeper expiration before the last sample was taken. It was most often difficult to get the patient to perform this deeper expiration without a deeper inspiration first, and this naturally disturbed the equilibrium. ESPERSEN has made use of the same modification in some of his experiments, but he has not given these values in the discussion on his results. Most often his values, calculated with the help of the sample at the end of deep expiration, are lower than the others, but often higher too. This latter fact might be explained by the patient just taking a deeper inspiration before the deep expiration. This modification will not be a generally practicable manner, but when able to be carried out, however, it gives very good results, as will be seen from the following example from a compensated hypertonic patient:

	CO <sub>2</sub>	C <sub>2</sub> H <sub>2</sub>	O <sub>2</sub>	Art.ven. O <sub>2</sub> diff.
Sample 2 normal expiration	4.80 %	5.33 %	16.11 %	70.5 cc/l
» 3 deep »	5.53 %	4.64 %	14.92 %	61.1 cc/l

If the arterio-venous oxygen difference from sample 2 is calculated with 4.64 as the value of the acetylene mean concentration instead of 5.23, the value of the arterio-venous O<sub>2</sub> difference will be 61.3 cc/l. Equilibrium thus exists. Parallel GROLLMAN determinations gave the following values: 59.6 cc/l; 58.6 cc/l; 60.1 cc/l. Average: 59.4 cc/l.

The author has, moreover, endeavoured to reduce the dead space, as far as is practically possible, by taking one or all the samples in the mouth on the conclusion of the expiration, by means of a test tube in the valve lengthened with a little capillary rubber tubing. When taking similar samples in the mouth with evacuated sample tubes, a systematic error may be imagined to exist on account of air being sucked in from the inspiration side of the valve. That this is not the case will be shown in the following experiment. A series of mouth samples were taken while breathing air at the end of expiration, firstly in evacuated sample tubes and secondly as fractioned samples in non-evacuated sample tubes. The carbon dioxide values in 6 fractioned samples were in the mean  $5.04 \pm 0.03$  %, in 6 samples in evacuated sample tubes  $5.09 \pm 0.04$  %, thus no difference ( $0.05 \pm 0.05$  %) that can be proved. Should room air be admixed, the carbon dioxide percentage must be lower in samples taken in evacuated sample tubes. Even with this technique, however, it is not always possible to get down the values of the arterio-venous difference to those obtained in simultaneous GROLLMAN determinations, besides which it is often difficult to have time to get sufficient quantities of samples. This method is not altogether satisfactory either.

#### Modification with 600 cc Depth of Respiration.

In consequence of the experiences above described, the author has finally modified the E. NIELSEN method in the following manner. The principle has been to assure a sufficiently great depth of respiration. The apparatus will be seen in fig. 3. Nos. 1, 2 and 3 are 3 spirometers with a capacity of about 20 liter, graduated in portions of 600 cc. They are each attached by means of rubber tubings to the four-way cock, C. L is a lever with which the 3 conveying rubber tubings can be simultaneously completely pressed together. The spirometers 2 and 3 contain the primary and the test mixtures respectively, and replace the rubber bag and the DOUGLAS bag in earlier apparatus. Spirometer 3 is furnished with a motor fan, and this produces homogeneous gas mixture previous to the experiment. Spirometer 1 contains air.

When determining the arterio-venous difference the patient is instructed to inspire somewhat more deeply than usual till he notices resistance, when he changes over to expiration, apart from this breathing is as usual. An assistant follows the volume in-

spired on the spirometers, and when the patient has received 600 cc, each inspiration is broken off by compressing the rubber tubings. First of all the patient must respire air from spirometer 1 in order to get into a regular and even state of breathing. Communication is then established with spirometer 2 containing the primary mixture (about 30 % acetylene), from which 2 inspira-

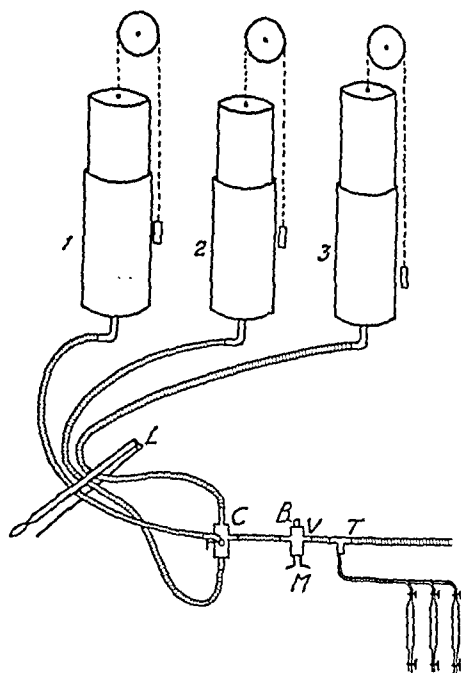


Fig. 3. Apparatus for the modified Nielsen method with 600 cc depth of respiration.

tions are made, after this inspirations are continued from spirometer 3 with the test mixture. The samples are taken according to the technique already described.

The advantages of this technique are obvious. The influence of the dead space is eliminated, as well as variations in the depth of respiration. There is no need to vary the primary mixture so much as before. It is sufficient to use 2 inspirations of primary mixture, and the right strength can generally be chosen after one preliminary experiment.

The drawbacks are, firstly more co-operation is required of the subject, secondly an assistant is required to measure the inspired volumes. This latter inconvenience can be overcome by means of an automatic clip arrangement, which is to be con-

structed. So far the method has been adopted for 2 decompensated patients as well as for a number of normal subjects, and it has not been difficult to teach the patients to breathe in the way required. There can be no talk of any hyperventilation which can increase the circulation, as the time for the test on normal subjects can be extended to 55—60 seconds without any noticeable rise in the values of the arterio-venous oxygen difference.

When comparing the GROLLMAN method with this modified E. NIELSEN method in normal subjects, determinations under standard conditions being made in close connexion with each other, the following results were obtained (Table 7).

Table 7.

	Art.ven. O <sub>2</sub> diff. according to GROLLMAN	Art.ven. O <sub>2</sub> diff. according to E. NIELSEN 600 cc mod.	Difference GROLLMAN—E. NIELSEN
Mean:	58.95 cc/l	57.95 cc/l	+ 1.00 cc/l
	± 2.096	± 1.944	± 2.990
n = 15			

Thus no difference is observed in the values according to the GROLLMAN and the modified E. NIELSEN method. As it must be considered an accomplished fact that the GROLLMAN method gives correct values in healthy subjects, the above comparison shows that really correct values are obtained by this modified E. NIELSEN method.

Statistical treatment of double determinations under standard conditions on normal subjects in the same way as before gave the following results (Table 8).

Table 8.

	I values	II values	Difference I—II
Mean . . . . .	63.64 cc/l	62.98 cc/l	— 0.66 cc/l
n = 16			± 1.060
			$\sigma_d = \pm 4.243$ cc/l
Standard deviation of a single determination . . . . .			$\sigma_t = \pm 3.000$ cc/l
Mean of I and II values: 63.31 cc/l			
Variation coefficient: 4.74 %.			

A source of error may be found to exist in the subject's manner of breathing. On two healthy subjects suspiciously high values were obtained in the arterio-venous oxygen difference, while the GROLLMAN values were about the normal. It proved



that these subjects had a strikingly slow respiration and that they made a long respiration pause after every expiration. In such circumstances the acetylene percentage at the end of an expiration cannot be presumed to represent the actual alveolar mean concentration, instead, this is probably considerably lower. The result will be too high a value for the arterio-venous oxygen difference. In one of the cases it was possible to repeat the experiments, and the subject was made to respire more rapidly without any pause in the expiration. Now the values were normal. The values in the latter case will be given (Table 9).

Table 9.

*Determinations of the arterio-venous  $O_2$  difference under standard conditions with and without pause after expiration,*

	E. N. mod. 600 cc GROLLMAN	
a) with slow respiration and a pause after expiration . . . . .	I 73.4 cc/l	59.2 cc/l
	II 83.1	58.8
b) with more frequent respiration and without any pause in the expiration . . . . .	I 59.0	61.5
	II 65.0	47.2
c) with more frequent respiration and without any pause in the expiration a day or two later	I 62.1	49.3
	II 65.9	57.3

Attention must be directed to this source of error, which seems to be relatively rare and easy to adjust. With dyspnoic patients there will be no risk of it being of any importance.

A metronome might sometimes prove a help when regulating the breathing. It is, however, a drawback, if the subject must concentrate on two things, firstly keeping a certain time when breathing, and secondly inspiring the volume measured. Hitherto it has proved quite satisfactory to let the subject determine the pace. Continued experience on this point is to be awaited.

## Other Methods.

The determination of *oxygen consumption* forms a special part in the determination of the cardiac output. It is only the arterio-venous oxygen difference that is determined by the acetylene experiment, and the cardiac output is obtained by dividing oxygen consumption by the arterio-venous difference.

During the first part of these investigations oxygen consumption was determined by means of a KROGH spirometer. In this apparatus the breathing resistance was sometimes troublesome for dyspnoic patients, besides which the curves were not always so even as were desirable. The measurement of the curves took place with an accuracy of  $1/2$  mm. An uncertainty of 1 mm will often have to be calculated with, which on a curve corresponding to 5 min. makes c. 8 cc oxygen pr. min.

Another disadvantage with the spirometer method is that the alveolar oxygen tension is higher than in normal breathing. The oxygen percentage in alveolar samples under spirometer breathing seems as a rule to be about 35 %, according to what the author has found when taking odd samples. If a considerable arterial oxygen unsaturation exists, the oxygen absorption under spirometer breathing may be greater than when the subject breathes under ordinary conditions. Generally speaking there does not seem to be any considerable degree of unsaturation in the case of congestive heart failure. BLUM (1942) found an average saturation of 91 % in such cases. The effect of these conditions may, moreover, be considered relatively constant during the 2—4 hours that the test in question took, and are in consequence of no importance when determining the changes.

The author subsequently passed on to the bag method introduced by DOUGLAS (1911). When adopting this method, the expiration air is collected into a DOUGLAS bag during a certain period of time, the composition of the mixed expiration air is determined and the volume of the air expired is measured. The same oxygen tension exists in the alveoli in this method as in ordinary breathing. It also allows of the calculation of the respiratory quotient, which is obtained by dividing the carbon dioxide difference by the oxygen difference.

The volume of the expiration air collected in the DOUGLAS bag was measured in such a manner that, when its contents had been thoroughly mixed by being knocked and banged about, the bag was emptied through a gas meter as evenly and completely as possible. To make sure, 2 samples have been taken from different portions each time the bag was emptied, and these were analyzed independently. There was good agreement as a rule, though not always, which indicates that the mixing was then not sufficiently complete. Two values of the oxygen consumption were calculated in every determination and henceforward

the mean of these two will be used in the calculations, thus avoiding errors of any importance.

The accuracy of the measurement of the volume was controlled in the following manner. A definite quantity of air, approximately corresponding to the volumes generally collected in the experiments was measured up in a big spirometer. This quantity of air was transferred into the empty bag, which was then emptied through the gas meter in the manner already described. 21 such experiments were made. The volume measured in the gas meter was 1.6 % too little in the mean, with a standard deviation of  $\pm 2.3$  %. Consequently no correction has been made. In any case it would be of no importance when judging the variations.

Table 10 shows a comparison between all the double determinations of the oxygen consumption according to the bag method of patients with congestive heart failure.

Table 10.

	I	II	Difference II-I
Mean . . . . .	244.5 cc/min.	248.7 cc/min.	+ 4.2 $\pm$ 1.42 cc/min.
n = 40			$\sigma_d = \pm 8.96$

Thus a statistically significant increase ( $2.96 \times$  standard error) amounting to 1.7 % is to be found from the value I to II. This slight increase, however, may be ignored. If the standard deviation of a single determination is calculated in agreement with this by the division of the standard deviation of the differences with  $\sqrt{2}$ , the following result is obtained:

$$\sigma_t = \pm 6.33 \text{ cc/min.}$$

The variation coefficient: 2.56 %.

This is naturally an approximate value and is somewhat too high, as the variation which is the result of the slight increase from value I to value II is included in it.

If 8 earlier double determinations according to the KROGH method are included, in which there is no statistical difference, a slight statistically significant increase from value I to value II of  $5.5 \pm 1.63$  cc/min. making 2.2 % is found. Even this difference will be considered negligible.

If all the values of the *cardiac output* in double determinations under standard conditions of decompensated patients are examined, the following result is obtained (table 11). There is a difference between the I and the II values.

Tabell 11.

*The cardiac output of decompensated patients, double determinations under standard conditions.*

	I values	II values	Difference II—I
Mean . . . . .	2.585 l/min.	2.629 l/min.	+ 0.045 l/min.
n = 46			± 0.0317
			$\sigma_d = \pm 0.2153$
Standard deviation of a single determination . . . .			$\sigma_t = \pm 0.1522$
Variation coefficient 5.84 %.			

The *heart rate* has been determined by fixing the time for a suitable number of pulsations, usually between 40 and 60, by means of a control clock. In cases with pulse deficit, the heart beats were counted with the help of auscultation. The *stroke volume* is obtained by dividing the cardiac output by the heart rate. In table 12 the values of the stroke volume in double determinations have been registered. No difference has been found here either between the I and the II values.

Table 12.

*The stroke volume of decompensated patients, double determinations under standard conditions.*

	I values	II values	Difference II—I
Mean . . . . .	36.15 cc	36.39	+ 0.25 cc
n = 44			± 0.546
			$\sigma_d = \pm 3.619$ cc
Standard deviation of a single determination . . . .			$\sigma_t = \pm 2.559$
Variation coefficient 7.05 %.			

As already mentioned, the values of the arterio-venous oxygen difference and consequently of the cardiac output and the stroke volume cannot be regarded as absolute. Even making allowance for this, the average cardiac output in the cases with congestive heart failure is considerably lower than in normal cases. The average values are probably less than 10 % too low. The average for the heart index, i. e. the cardiac output per m<sup>2</sup> body surface was 1.5, whereas the normal range according to GROLLMAN (1932) is  $2.2 \pm 0.2$ . As there is often an increase in the standard metabolism in congestive heart failure, average standard metabolism in this material being + 21 %, the value of the arterio-venous oxygen difference gives a better comprehension of the magnitude of the blood stream. GROLLMAN gives a normal range of 55—67 cc/l. The average for the cases of congestive

heart failure is considerably higher than normal, even if the figure, 98 cc/l may be 10 % too high.

The blood pressure was measured by means of an ordinary blood pressure cuff with a metal manometer, auscultation being used. The reading was taken with an accuracy of 5 mm Hg. The manometer was controlled by comparing it with a methyl-iodide manometer according to LILJESTRAND and ZANDER (1928).

### Clinical Examination of Patients.

The examination of patients included an ordinary physical and a roentgenological examination, an electro-cardiogram, the determination of venous pressure and circulation time.

The roentgenological examination was made by the method adopted at the Serafimer Hospital since 1935, and which was described by LILJESTRAND, LYSHOLM, NYLIN and ZACHRISSON (1939) and by JONSELL (1939). The heart volume is calculated with the help of roentgenograms in two planes at right angles. The calculation is based on ROHRER and KAHLSTORF's formula. The heart volume is related to the body surface, as introduced by LYSHOLM, NYLIN and QUARNÅ in 1934. The normal heart volume seems to vary between 250 and 490 cc pr. square meter of body surface, in the majority of normal people this figure lies between 300 and 400 cc pr. sq. m. Variations of less than 50 cc cannot be determined by this method.

The venous pressure was estimated in the direct bloody manner with the help of a vertical glass tube in which the blood rises with no additional fluid, as has been described by TAYLER, THOMAS and SCHLEITER (1930). According to these authors, normal venous pressure varies between 4 and 10 cm. The instrument, described by COHEN (1936), was used. The apparatus was moistened with heparine solution to prevent coagulation. COHEN gives a normal range of 6—12 cm for the venous pressure.

The circulation time was determined by the decholin method introduced by WINTERITZ, DEUTSCH and BRÜLL (1931). According to these authors, the normal circulation time from arm to tongue is 8—14 seconds. TARR, OPPENHEIMER and SAGER (1933) give 10—16 seconds as the normal values with a mean value of 13 seconds, NYLIN and MALMSTRÖM (1942) 8—21 seconds, mean value 13 seconds.

## General Conditions of the Experiments.

The subjects were previously trained in and got accustomed to the methods used in connexion with the preliminary experiments. All the examinations have been carried out under so-called standard conditions, as has usually been the case in other investigations on the cardiac output. The examinations were carried out before noon, and the patients had previously fasted for about 14 hours and were in bed. They were taken to the laboratory in bed and rested for  $\frac{3}{4}$ —1 hour after the transport. Then 2 initial determinations were taken, with an interval of about 20 minutes. Every determination included the counting of the heart rate, the measurement of the blood pressure, the determination of the oxygen consumption as well as the arterio-venous oxygen difference, all of this taking about 10 minutes to carry out. There was an interval of at least 30 minutes between two successive acetylene experiments, which should be reassuring, as according to GROLLMAN 12—15 minutes is sufficient for acetylene to disappear from the body of a normal person. When these two basic determinations were concluded, the drug in question was injected and fresh determinations taken at suitable intervals.

As no difference has been found between these two initial determinations, it seems justifiable to let the average value of these form the basis when judging the effect. As has already been pointed out, however, there is a difference between the two values with regard to the oxygen determination. This difference being so slight and only existing in this function, the same method of calculation has been applied here. In each drug test the difference between the values after the injection and the average of the basic determinations have been calculated, after which these differences have been worked out statistically. As the determinations both before and after the drug injection were carried out on the same subjects and at one and the same time, this method of calculation is permissible and advantageous.

As a rule the patients were not treated with digitalis until the experiments were completed. If cardiac glucosides were considered necessary, strophanthin was given, its action being of short duration. On this basis it may be considered, that the patients were not under the influence of any drug when the initial values were taken.

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## PART II.

### Effect of Drugs on the Cardiac Output.

#### Effect of Digitalis and Strophanthin on the Cardiac Output.

##### Previous Investigations.

##### Animal Experiments.

In heart-lung preparations the heart glucosides diminish the size of the heart, and when insufficiency of the heart has caused a fall in the cardiac output, this is increased by therapeutic doses of strophantin (BIJLSMA and ROESSINGH, 1922, ANITSCHKOW and TRENDLENBURG 1928) and digitalis (COHN and STEELE, 1932). In intact animal (dogs) it was found that large doses of digitalis caused a decrease in the cardiac output (HARRISON and LEONARD, (1926), COHN and STEWART, (1928), DOCK and TAINTER, 1930). A decrease in cardiac output and heart size was found by COHN and STEWART (1928) in dogs with heart enlargement due to experimental insufficiency of the mitral valve. In dogs with artificial auricular fibrillation and consequently diminished cardiac output and dilated heart, these authors found an increase in cardiac output and decrease in cardiac size by digitalis (STEWART and COHN 1932).

##### Effect on Normal Human Subjects.

BURWELL, NEIGHBORS and REGEN (1927) studied the action of digitalis on 4 normal subjects, using the carbon dioxide method according to FIELD, BOCK, GILDEA and LATHROP (1924). An initial dose of 1 gram digitalis powder was given by mouth, the following days doses of 0.2—0.8 gram until nausea appeared. They found a decrease in cardiac output, comparable to that found by HARRISON and LEONARD in dogs. STEWART and COHN (1932) using

GROLLMAN's original acetylene method studied the effect of 0.8—1.0 gram of digitan (Merek) given by mouth in a single dose or within 10 hours. They found a decrease in cardiac output, which began within 4—12 hours and was most pronounced after 24 hours, when the cardiac output was reduced to 60—85 % of the initial amount. The oxygen consumption remained constant, the decrease in cardiac output being combined with increase in arterio-venous oxygen difference. The effect wore off from within 48 hours to 3 weeks. Some of the subjects complained of lassitude and dyspnea on exertion. One subject with the most pronounced and lasting effect got severe dyspnea and cardiac pain. There were changes in the T waves of the electrocardiograms, in one case still present 38 days later. LARSEN, NEUKIRCH and NIELSEN (1936) and NEUKIRCH (1938) made similar experiments, when they investigated the effect of a single dose of 1.0 gram of folium digitalis or 2 doses of 0.6 and 0.8 gram after an interval of 24 hours. They found a decrease in the cardiac output in 6 out of 8 healthy subjects of 10—27 % of the initial value, and there was no change in the remaining two. In an experiment with strophanthin (0.5 mg. intravenous) they found a decrease of 20 % after 1 hour.

All these investigations seem to point to a decrease in the cardiac output. DOCK and TAINTER (1930) found a decrease in venous pressure in the above mentioned animal experiments, and this was confirmed by RYTAND (1933), in experiments on healthy human subjects with the same large doses. These authors consider that the diminution of the cardiac output produced by digitalis is due to a peripheral action of the drug. On the other hand, STEWART and COUX (1932) found no significant effect on the venous pressure, and consider that the decrease in cardiac output is due to a direct action on the heart, resulting in diminution of the heart size. Objections not to be overlooked, however, can be made to the large single doses used in the above mentioned works, for a toxic injury may be the result (WEESE 1936, 1939). These so-called "full therapeutic doses", founded on HATCHER's (1912) experiments on cumulation, were introduced by EGGLESTON (1915), and seem still to be generally adopted in American practice, with a slight modification by ROBINSON, WHITE, EGGLESTON and HATCHER (1924). FROMHERZ and BAUER (1933, 1934, 1937) again revised the cumulation problem, and concluded that the cumulative effect of large doses of digitalis are due to



toxic injury and that the glucoside itself will have been destroyed within 48 hours. If STEWART's and COHN's aforesaid experiments with lasting effect and severe symptoms in the subject are considered in the light of these works, a toxic injury may be regarded as fairly likely. In a later paper LILJESTRAND and NYLIN (1941), could not find any effect on the cardiac output in healthy subjects after small doses, which are nevertheless therapeutically effective when there is heart insufficiency. They gave intravenous injections of 1—1.5 cc Digitotal (1 cc of which corresponds to 0.1 gr. *Folium digitalis*), but no determinations were made later than 1 hour after the injection.

### Effect in Cases of Cardiac Disease in Man.

Using their modified ethyl iodide method, STARR, GAMBLE, MARGOLIER, DONAT, JOSEPH and EAGLE (1937) found no constant effects from digitalis on the cardiac output of patients with cardiac disease without congestive failure. STEWART and COHN (1932) in the paper mentioned above, using the same technique and dosage, also presented studies of digitalis action in patients with congestive heart failure. They found an increase in the cardiac output. FRIEDMAN, CLARK, RESNIK and HARRISON (1935) criticized these investigations considering the original GROLLMAN method too unreliable in such cases. The same criticism may also be directed to experiments by GRASSMAN and HERZOG (1931), in which they investigated the immediate effect of strophanthin after intravenous injection. FRIEDMAN et al. studied the problem anew, using the "three sample" acetylene method, and concluded that clinical improvement produced by digitalis may be associated with an increase, a decrease or no change in the cardiac output. STEWART, CRANE, DEITRICK and THOMPSON (1938) studied the action of digitalis in compensated heart disease of rheumatic, arteriosclerotic, hypertensive and syphilitic origin. They gave 1.6—1.8 gram of digitalis within 24 hours and determined the cardiac output before and in the following days, using the "three sample" technique. They found no constant effect either on the cardiac output or on the venous pressure. STEWART, DEITRICK, CRANE and WHEELER (1938) using the same method concerning patients with uncompensated heart disease, found in these cases an increase in cardiac output following digitalis therapy. They emphasize that the digitalis effect common to

all hearts is the decrease in the cardiac size, which they interpret as an effect on tone. They think that the amount of the cardiac output which results from this action is due to the initial size of the heart. HARRISON (1939) will not accept their conclusions concerning the cardiac output and refers to his and his co-workers' paper mentioned above (1935). He finds *inter alia* that they are inclined to attach importance to changes in cardiac output which are well within the error of the method used.

Judging from literature there seems to be unanimity of opinion on that large doses of digitalis cause a decrease of the cardiac output in a healthy heart, while some authors who use comparatively small doses find no effect. In compensated heart disease no constant effect has been observed. Concerning the effect in cases of congestive heart failure, which is naturally of the greatest interest, there are, on the other hand, different opinions. The inconsistent results may very well be due to the unsuitability of the method used in these cases. The usual manner of giving large single doses of digitalis by mouth, and making determinations before and after digitalis on different days, must be considered rather unsuitable, partly on account of the size of the dose (see above), and partly because the variations from day to day, which can be fairly considerable, may have an effect on the result.

### The Author's Experiments on the Action of Digitalis.

In the author's studies of the action of digitalis determinations of the cardiac output were made,  $1\frac{1}{2}$  and 2 hours after the intravenous injection of 2 cc of the digitalis preparation Digiton (Leo), which is generally used for injection purposes at the Royal Serafimer Hospital. Digiton contains both purpurea and lanata glucosides and is so standardized that 1 cc corresponds to 0.13 gram of the international standard digitalis powder (1936). Some determinations were also made after 1 and  $2\frac{1}{2}$  hours, respectively, but as the greatest effect seemed to appear  $1\frac{1}{2}$  hours after the injection the author concentrated on the latter determinations.

### Experiments on Normal Subjects.

It seemed desirable to apply the above mentioned doses and intervals on some healthy subjects, earlier investigations not being entirely comparable in these respects. The author has

Table 13.

*The effect of Digitalis in normal cases. Mean of 5 experiments in 4 subjects.*

*Dose 2 cc Digilon = 0.26 gram of the international digitalis powder standard, given intravenously.*

Function	Before the injection $\frac{I + II}{2}$	1½ hour after the injection III	Diff. $\frac{I + II}{2}$ III—	2 hours after the injection IV	Diff $\frac{I + II}{2}$ IV—
Art. ven. ....	67.08	65.56	- 1.52	66.16	- 0.92
O <sub>2</sub> -diff cc/lit.			± 3.676		± 2.42
Change %.....			0		0
Cardiac output l/min .....	3.218	3.414	+ 0.196	3.422	+ 0.204
			± 0.1933		± 0.1770
Change %.....			0		0
Stroke volume cc/beat.....	57.00	65.76	+ 8.76	66.66	9.66
			± 2.850		± 4.039
			(+ 15 %)		(+ 17 %)
Change %.....			- 4.92	52.14	- 5.26
Heart rate .....	57.40	52.48	± 1.315		± 1.603
			- 9 %		- 9 %
Change %.....					

therefore made 5 experiments on 4 subjects with no sign of circulation disease (2 medical students and 2 patients with lumbago and sciatica). The last modification of the NIELSEN method with 600 cc respiration depth was used. The averages are given in Table 13. No significant effects were obtained on the arterio-venous difference and the cardiac output. No changes were found in the blood pressure. The stroke volume was increased by about 15 %, the heart rate decreasing by about 9 %.

As the number of experiments is rather small, the three series of differences (between the initial values, between the III value and the average of the initial values and between the IV value and same average) were treated statistically by analysis of variance. [Snedecor's F-test, (1938) quoted from BONNIER and TEDIN (1940)]. Thus consideration is taken to the number of values. The result concerning the cardiac output figures was  $P > 0.2$ ,  $P$  indicating the degree of probability that the differences are due to mere chance. The increase of stroke volume after 1½ hours was not found to be significant ( $0.2 > P > 0.05$ ).

P being of this magnitude, it is possible that further experiments would show a significant change of the stroke volume.

This is in agreement with the results of LILJESTRAND and NYLIN.

### Experiments on Patients with Congestive Heart Failure.

*Results with the Grollman method.* The GROLLMAN three sample technique was used in digitalis experiments on six cases, nos. 1—6 (Table 25, page 65). The method as applied by the author has already been criticised in a previous chapter (page 16—17), and no great importance will be attached to these results. The number of experiments being small, analysis of variance has been applied to the values of the cardiac output. The increases after 1, 1½ and 2 hours, respectively, have been compared with the differences of the corresponding initial values.

After ½ hour there was no trace of effect. After 1 hour (3 experiments) an insignificant increase of 14 %, ( $P > 0.2$ ) was found. After 1½ hours (4 experiments) a statistically probable increase of 21 % ( $0.01 > P > 0.05$ ) was observed, after 2 hours (5 experiments) 19 % ( $P = 0.05$ ).

These results suggest that digitalis causes an increase in the cardiac output in congestive heart failure, and they support the figures gained by the NIELSEN method, which are now to be reported.

*Results with the Nielsen Method.* The effect of digitalis was studied in 13 experiments on 12 decompensated patients with the help of the NIELSEN method, modified as described on pages 25—28. In table 14 the values of the arterio-venous oxygen difference, the cardiac output and stroke volume are given. Data on the patients are to be found in table 25. As will be seen, the origin of the heart disease was different (vitium org. cordis, hypertonia, cardiosclerosis). All of them had signs of decompensation, though in different degrees. The cases are too few to allow of any further analysis of smaller groups.

In case 8 the possibility of a congenital heart disease with septum defect was discussed. In such cases the cardiac output cannot be determined by the acetylene methods. There were, however, no positive signs of a septum defect, and the results obtained were reasonable. It may be of interest that a monozygotic twin sister did not show any clinical or roentgenological signs of heart disease. There seems to be no reason to discard this case.



By treating the differences statistically, the following results are obtained. Table 15.

*The cardiac output.* One hour after the injection there was an average increase of  $+0.383 \pm 0.0854$  l/min., the mean of the corresponding initial values being 1.970 l/min.<sup>1</sup> The number of experiments being only four, the differences between the initial values on the one hand, and between the 1 hour values and the average of the corresponding initial values on the other, have been treated statistically by analysis of variance. The increase is found to be statistically probable ( $0.05 > P > 0.01$ ). These figures suggest that there is an effect on the cardiac output already 1 hour after the injection.

After  $1\frac{1}{2}$  hours a significant increase of 19 % was established, after 2 hours 15 % and after  $2\frac{1}{2}$  hours 8 %. As determinations after  $2\frac{1}{2}$  hours were made in only 4 cases, analysis of variance has been applied, and by means of this the increase is found to be significant ( $P < 0.001$ ).

The experiment on subject no. 13 will be of special interest. It was a case of mitral stenosis and insufficiency, with pronounced decompensation and a comparatively slow heart rate, 52 per min. at the time of the digitalis experiment. Owing to this slow rate the patient was at first treated with diuretics and no digitalis. After a fortnight's treatment there were still signs of decompensation though in a lesser degree. In the digitalis experiment there was found an increase in the cardiac output from 2.32 l/min. to 2.59 l/min. after 2 hours and to 2.58 l/min. after  $2\frac{1}{2}$  hours. As the variation coefficient for the determinations of the cardiac output was found to be 5.81 % (page 37), both increases are about twice the standard deviation.

The patient then improved considerably while undergoing treatment with digitalis and diuretics, though he was not quite free from symptoms when leaving the hospital. This case illustrates that digitalis may have a favourable action on cardiac insufficiency in cases with a slow heart rate, which has been questioned in Anglo-Saxon literature.

*The arterio-venous oxygen difference.* A decrease of 15 % was found  $1\frac{1}{2}$  hours after the injection, of 13 % after 2 hours and of 7 % after  $2\frac{1}{2}$  hours. The last increase was only statistically probable. Analysis of variance confirmed this,  $0.05 < P < 0.01$ .

*The stroke volume.* A considerable increase of 34 % was established  $1\frac{1}{2}$  and 2 hours after the injection, while after  $2\frac{1}{2}$  hours no significant change was to be found. The largest percentual increase took place in case no. 23, where the very low stroke

<sup>1</sup> These values are not given in Tables 13 and 14.

Table 15.  
*The effect of digitalis in congestive heart failure on various functions. Dignon 2 cc = 0.24 gram of the international digitalis standard were given intravenously. I and II are initial values before injection, III the values  $1\frac{1}{2}$  hours after, IV the values 2 hours after and V the values  $2\frac{1}{2}$  hours after the injection.*

Function	Mean of the averages of initial values $\frac{I+II}{2}$	Mean of the differences $\frac{III - \frac{I+II}{2}}{2}$	Change $1\frac{1}{2}$ hours after injection Percentage	Mean of the averages of initial values $\frac{I+II}{2}$	Mean of the differences $\frac{IV - \frac{I+II}{2}}{2}$	Change 2 hours after injection Percentage	Mean of the averages of initial values $\frac{I+II}{2}$	Mean of the differences $\frac{V - \frac{I+II}{2}}{2}$	Change $2\frac{1}{2}$ hours after injection Percentage
Art.-ven. .... O <sub>2</sub> difference cc/l	95.19 n = 12	-14.68 $\pm 3.620$ n = 12	-15 %	97.83 n = 11	-12.88 $\pm 3.230$ n = 11	-13 %	93.25 n = 4	-6.15 $\pm 2.323$ n = 4	-7 %
Oxygen consumption cc/min.	249.6 n = 12	+5.1 $\pm 6.19$ n = 12	0	243.9 n = 13	+2.0 $\pm 3.01$ n = 13	0	302.20 n = 4	+3.1 n = 4	0
Cardiac output ..... l/min.	2.712 n = 12	+0.518 $\pm 0.0816$ n = 12	+19 %	2.538 n = 11	+0.370 $\pm 0.038$ n = 11	+15 %	3.015 n = 4	+0.233 $\pm 0.017$ n = 4	+8 %
Stroke volume..... cc	34.23 n = 11	+11.59 $\pm 1.518$ n = 11	+34 %	31.20 n = 10	+10.56 $\pm 1.377$ n = 10	+34 %	35.35 n = 4	+7.18 $\pm 3.895$ n = 4	0
Heart rate .....	85.12 n = 11	-11.12 $\pm 2.011$ n = 11	-13 %	87.52 n = 10	-13.67 $\pm 2.152$ n = 10	-16 %	85.92 n = 4	-11.50 $\pm 1.363$ n = 4	-12 %

volume of 15 cc was nearly doubled. In case no. 19 the heart rate was so rapid and irregular that the counting was given up.

The heart rate was decreased 13 %, 16 % and 12 % after  $1\frac{1}{2}$ , 2 and  $2\frac{1}{2}$  hours respectively.

In the oxygen consumption no significant changes were obtained in this material. In case no. 5 there was an increase from 225 cc/min. to 259 cc/min., this being the greatest increase observed. Cases nos. 3, 9, 15, 21 also had increases of between 14—22 cc/min., at different times. NYLIN (1938) in cases with severe decompensation and liver enlargement found a momentary increase of the oxygen consumption after large intravenous doses of digitalis. The increase was not observed in cases with liver cirrhosis. NYLIN used larger doses (3—5 cc Digiton), and all his cases had pronounced congestive heart failure, thus making his experiments not entirely comparable with those of the author.

There were no significant changes either in the systolic, the diastolic or the mean arterial blood pressure.

As has been previously pointed out (page 30) in connexion with the effect of the dead space, the values of the arterio-venous oxygen difference cannot be regarded as absolute, although the error is probably not great in most cases. The decrease in the values of the arterio-venous oxygen difference might possibly have been caused by a tendency to increased depth of respiration after the digitalis injection. If so, there should be a tendency to an increase of the carbon dioxide percentage in the samples after the injection. The difference between the carbon dioxide percentage in the samples before and those after the injection have been treated statistically in the same way as when

judging the different functions. The difference  $\text{III} - \frac{\text{I} + \text{II}}{2}$  was  $+0.091 \pm 0.0417\%$ , difference  $\text{IV} - \frac{\text{I} + \text{II}}{2}$  was  $-0.008\%$ . No

significant changes are to be found. In the ventilation and the respiratory quotient there were likewise no significant changes. Thus there seem to be no changes in the respiration to which the decreased values of the arterio-venous oxygen difference can be attributed.

The results may be summarized as follows. Digitalis, given intravenously has been found to cause a momentary moderate increase in the cardiac output in congestive heart failure. This increase was found in every case tested; it seemed to be most



Table 16.

*Effect of g-strophanthin in 9 normal subjects, 9 experiments,  
 $\frac{1}{4}$  mg. g-strophanthin was given intravenously.*

Functions	Mean of the averages of the initial values.	Mean of the values $\frac{1}{2}$ hour after the injection	Mean of the differences	Mean of the values 1 hour after the injection	Mean of the differences
	$\frac{I+II}{2}$	III	$III - \frac{I+II}{2}$	IV	$IV - \frac{I+II}{2}$
Art.-ven. ....	62.53	63.90	+ 1.37	67.67	+ 5.13
O <sub>2</sub> difference cc/l.			$\pm 1.417$		$\pm 3.221$
Change .....			0		0
Oxygen consumption cc/min.	222.9	223.1	+ 0.2	229.2	+ 6.3
Change .....			0		$\pm 4.52$
Cardiac output .... l/min.	3.601	3.561	- 0.040	3.56	- 0.040
Change .....			0		0
Stroke volume .... cc.	62.88	63.89	+ 1.01	64.05	+ 1.17
Change .....			0		0
Heart rate .....	58.30	56.21	- 2.09	57.02	- 1.28
Change .....			$\pm 0.745$		$\pm 0.639$
			(-3 %)		0

marked after  $1\frac{1}{2}$  hours and was still present after  $2\frac{1}{2}$  hours though less pronounced. It was combined with a decrease in the arterio-venous difference and an increase of the stroke volume.

### The Author's Experiments on the Action of Strophanthin.

When studying the effect of strophanthin, determinations were made 20—30 mins. and 50—60 mins. after the intravenous injection of  $\frac{1}{4}$  mg. g-strophanthin. This was chosen on account of its being a well defined crystalline substance and it keeps better than the amorphous k-strophanthin.

### Experiments on Normal Subjects.

The results of 9 experiments on 9 normal subjects (patients with sciatica or lumbago and with no signs of any circulatory disease, as well as medical students) are given in table 16. The

Table 17.

*The effect of Strophanthin in Congestive heart failure.  
Dose 0.25 mg g-strophanthin.*

Patient no.	Initials. Date of experiment	Before inj. I, II			III 20-30 min. after inj.			IV 50-60 min. after inj.		
		O <sub>2</sub> -Ven. cc/lit.	Art.-Ven. output lit./Min.	Cardiac output cc/beat.	O <sub>2</sub> -Diff. cc/lit.	Art.-Ven. output lit./Min.	Cardiac output cc/beat.	O <sub>2</sub> -Diff. cc/lit.	Art.-Ven. output lit./Min.	Cardiac output cc/beat.
7	A. W. 17/2-41 ..	108.0 92.8	2.63 3.06	29.2 34.0	83.4	3.46	38.4	85.4	3.47	38.5
10	J. H. L. 25/2-41	77.3 78.0	3.94 3.85	55.0 49.4	74.4	4.25	59.0	—	—	—
11	S. A. 24/3-41 ...	106.7 110.3	2.58 2.52	32.2 31.6	91.2	2.93	39.0	101.4	2.62	36.4
12	G. F. 3/3-41 ...	— 73.3	— 3.70	— 49.3	68.9	4.01	55.7	78.9	3.32	46.1
13	T. B. 1/3-41 ...	100.4 95.9	1.86 2.25	30.5 35.7	94.6	2.33	45.7	99.0	2.33	41.6
14	J. S. 27/10-41 ..	88.0 87.1	2.54 2.66	31.5 32.8	79.3	2.80	35.8	71.0	3.12	40.2
17	F. B. 14/11-41..	92.7 80.3	2.39 2.83	31.9 39.3	90.3	2.52	38.6	89.3	2.54	39.6
18	A. S. 17/12-41 ..	74.0 68.5	4.38 4.57	52.3 55.5	67.5	4.69	55.5	70.3	4.41	53.5
20	M. L. 13/2-42 ..	105.0 101.3	2.37 2.61	31.1 30.8	84.3	3.06	39.5	102.4	2.60	31.6
21	J. S. 10/4-42 ...	91.2 97.6	2.99 2.87	4.10 39.4	93.0	3.13	44.3	99.9	2.56	42.6
22	F. S. 13/4-42...	110.6 120.9	2.12 2.04	26.8 27.5	97.3	2.59	36.9	110.5	2.63	38.8
23	M. K. 7/5-42 ..	96.1 115.4	2.27 1.86	19.3 16.9	95.5	2.46	23.6	114.5	2.04	19.8
24	V. W. 13/5-42 ..	115.9 124.9	1.90 1.83	20.7 18.9	119.3	1.86	23.0	110.0	2.04	24.8
24	*V. W. 13/5-42 .	109.6 114.6	1.73 1.64	21.1 18.7	100.6	1.95	28.0	96.5	2.08	30.0
25	J. W. 2/6-42 ..	117.6 111.8	1.76 1.92	36.5 34.2	112.6	1.90	49.6	100.0	1.91	47.5

\* In this experiment 0.5 mg g-strophanthin was given why it is not included in the calculations.

Table 18.

The effect in congestive heart failure of g-strophantin on various functions.  $\frac{1}{4}$  mg. was given intravenously. I and II are the initial values before the injection, III the values  $\frac{1}{2}$  hour (20—30 mins.) after, IV the values 1 hour (50—60 mins.) after.

Functions	Mean of the averages of the initial values	Mean of the differences	Change $\frac{1}{2}$ hour after the injection Percent- age	Mean of the averages of the initial values	Mean of the differences	Change 1 hour after the injection Percent- age
	$\frac{I+II}{2}$	$III - \frac{I+II}{2}$		$\frac{I+II}{2}$	$IV - \frac{I+II}{2}$	
Art.-ven. ....	96.99	- 7.76	- 8 %	98.48	- 5.46	- 6 %
O <sub>2</sub> difference cc/l.	n = 14	$\pm 1.974$ n = 14		n = 13	$\pm 2.481$ n = 13	
Oxygen consump- tion ....	252.4	+ 5.1	(+ 2 %)	252.4	+ 2.9	0
cc/min.	n = 14	$\pm 2.13$ n = 14		n = 14	$\pm 4.14$ n = 14	
Cardiac output ..	2.716	+ 0.283	+ 10 %	2.625	+ 0.112	0
l/min.	n = 14	$\pm 0.0514$ n = 14		n = 13	$\pm 0.0880$ n = 13	
Stroke volume...	35.11	+ 6.65	+ 19 %	33.79	+ 4.75	+ 14 %
cc.	n = 14	$\pm 0.976$ n = 14		n = 13	$\pm 1.273$ n = 13	
Heart rate .....	79.51	- 5.98	- 8 %	79.51	- 6.76	- 9 %
per min.	n = 14	$\pm 2.034$ n = 14		n = 14	$\pm 1.603$ n = 14	

Grollman method was used in three of them, which were carried out at an earlier stage, the modified NIELSEN method with 600 cc depth of respiration in the remaining six. As these two methods have been shown to give the same results in healthy subjects (page 33), the determinations have been treated together statistically. No significant changes occurred.

### Experiments on Patients with Congestive Heart Failure.

The effect of g-strophantin was studied in 14 cases of congestive heart failure with the help of the modified NIELSEN method as described on pages 25—28. The figures for the arterio-venous oxygen difference, the cardiac output and the stroke volume are given in table 17 and the results may be seen in table 18. In the arterio-venous oxygen difference there was a decrease of 8 % after  $\frac{1}{2}$  hour and 6 % after 1 hour.

The cardiac output was increased by 10 % after  $\frac{1}{2}$  hour, while after 1 hour there was no significant increase. In subject no. 24 the experiment was repeated with the double dose ( $\frac{1}{2}$  mg.), resulting in a more pronounced effect than with  $\frac{1}{4}$  mg. dose. (Table 17.) These values are not included in the calculation.

The stroke volume was increased by 19 % after  $\frac{1}{2}$  hour and by 14 % after 1 hour.

The heart rate decreased by 8 % after  $\frac{1}{2}$  hour and by 9 % after 1 hour.

Patient no. 11, a case with aortic and mitral insufficiency, had an atrio-ventricular block I with a conduction time of 0.38 sec. and for this reason he was treated with xanthines and mercurial diuretics and no digitalis. As there was no improvement he was given g-strophanthin. In the experiment on March 24, 1941 there was a rise in the cardiac output from 2.55 to 2.93 lit./min.  $\frac{1}{2}$  hour after the injection. After 1 hour the cardiac output fell to 2.62 lit./min., and there seemed to be a transitory aggravation of the conduction disturbance, as it was found by auscultation that the heart sounds corresponding to one beat disappeared 3 times a minute. This could not be verified in an electrocardiogram taken about 15 mins later. While under strophanthin treatment the patient at first improved, but then got worse and did not react to any treatment. In the electrocardiogram there were at times Wenckebach's periods. Finally the patient died suddenly on April 22.

When examining the carbon dioxide values in the same manner as in the digitalis experiments (page 49), the following results were obtained. The difference  $\text{III} - \frac{\text{I} + \text{II}}{2}$  was

$+ 0.044 \pm 0.063$ , the difference  $\text{IV} - \frac{\text{I} + \text{II}}{2}$  was  $+ 0.012 \pm 0.070$ ,

neither was there any change in the ventilation nor in the respiratory quotient.

Thus g-strophanthin and digitalis have been proved to have principally the same effect in congestive heart failure, though as was to be expected, the effect of strophanthin appeared more quickly and disappeared sooner than was the case with digitalis.

It may be of interest that the doses of 2 cc digiton and  $\frac{1}{4}$  mg. g-strophanthin correspond approximately to each other with regard to their potency, which is ascertained by biological standardization. The guinea-pig method of KNAFFL-LENZ (1926) is now used to a great extent for this purpose. 1 gram of the international digitalis powder standard (1936) contains 55—60 guinea-

pig units, 1 mg. g-strophanthin 45—50 guinea-pig units, according to RYDIN and GOLDBERG (personal communication). 1 cc Digiton corresponds to 0.13 gr. of the international digitalis powder standard (1936). 2 cc Digiton thus contains 14—16 guinea-pig units,  $\frac{1}{4}$  mg. g-strophanthin 11—13 units. The average increase in cardiac output caused by digitalis was 19% at most, by g-strophanthin 10 %. It seems that these figures suggest rather good agreement between the effect on the cardiac output in congestive heart failure in man, and the potency of digitalis and strophanthin ascertained by standardization on animals. It is possible that the maximum effect of g-strophanthin appears sooner than after  $\frac{1}{2}$  hour and thus the agreement in reality may be even better.

## The Effect of Xanthines on the Cardiac Output

### Previous Investigations.

#### Animal Experiments.

FLAUM and RÖSSLER (1933) investigated the effect of caffeine, theobromine and theophylline in heart-lung preparations which were injured and made insufficient by barbiturates, chloroform, carbon dioxide or long duration of the experiment. They found a considerable increase in the cardiac output and the stroke volume, which increase seemed to be independent of changes in the coronary circulation, and which was ascribed to a direct effect on the myocard. The doses were rather large (0.05 gram theobromine sodium-acetate and theophylline sodium-acetate, 0.2 gram caffeine with sodium benzoate). BOCK and BUCHHOLTZ (1920) in dogs with intact circulation found no change in the cardiac output from caffeine.

#### Experiments on Normal Human Subjects.

MEANS and NEWBURGH (1915), using the mitrous oxide method studied the action of 0.3—0.42 gram of caffeine sodium salicylate. They found an increase in the cardiac output without a corresponding increase of the oxygen absorption. In experiments during work, no conclusive effect was found. However, their experiments were few, and in some of them there was an interval of several weeks between the initial determinations and those under drug action

for which reason they are not fully convincing. GROLLMAN (1932) concludes that small doses of caffeine are without effect on the cardiovascular system. Larger doses (0.5—1.0 gram) cause, in most cases, a rise in oxygen consumption, an increase in the arterio-venous oxygen difference and a slight rise in the cardiac output. NEUTHARD and HOEN (1937) investigated the effect of caffeine, theobromine and theophylline in one subject, using the GROLLMAN method and found very great increases in the oxygen consumption and the cardiac output. Their values, however, are quite obviously inconsistent, the experiments were not made under standard conditions and the subject was apparently extremely sensitive to caffeine. Therefore these results cannot be taken into consideration.

### Experiments on Patients with Heart Disease.

FRIEDMAN, RESNIK, CALHOUN and HARRISON, 1935, with the three sample GROLLMAN method investigated the effect of diuretics on the cardiac output of patients with congestive heart failure. They made single determinations before and after the oral administration of from 1.5—0.9 grams of theophylline daily for two days. No constant effect on the cardiac output was observed. In this manner, however, it is rather the effect of loss of oedema than the direct effect of theophylline on the cardiac output that is elucidated. STARR et al. 1937 in the paper mentioned on page 42 also studied the effect of caffeine 0.5 gram and of 0.48 gram theophylline ethylendiamine (0.34 gram theophylline), given in intramuscular injection on patients with compensated heart disease. They made determinations before and 20—40 mins. after the injection. The average values showed an increase in cardiac output and stroke volume, significant only for theophylline. The oxygen consumption was not changed in most of the cases, in one there was a marked increase.

### The Author's Experiments.

Theophylline alone was studied, in the form of theophylline diaethanolamine. This solvent seems to be the most suitable, having no pronounced effect itself on blood pressure and respiration, while ethylendiamine causes a fall in blood pressure and stimulates respiration (LÜRMAN and LAUER 1933, FREY and HESS

Table 19.

The effect of theophylline diaethanolamine in normal subjects. 1 cc Deriphyllin = 0.20 gram theophylline was given intravenously. I and II are the initial values before the injection, III the values 15—25 mins. after, IV the values 40—50 mins. after the injection.

Functions	Mean of the averages of the initial values $\frac{I+II}{2}$	Mean of the differences $III - \frac{I+II}{2}$	Change 15—25 mins. after injection Percentage	Mean of the averages of the initial values $\frac{I+II}{2}$	Mean of the differences $IV - \frac{I+II}{2}$	Change 40—50 mins. after injection Percentage
Art.-ven. ....	60.20	-2.93	0	60.22	-0.88	0
O <sub>2</sub> difference cc/l	n = 4	$\pm 5.79$ n = 4		n = 5	n = 5	
Oxygen consumption cc/min.	223.7	+29.5 $\pm 2.32$ n = 6	+11 %	223.7	+26.5 $\pm 5.16$ n = 6	12 %
Cardiac output ... l/min.	3.858	+0.770 $\pm 0.5554$ n = 4	0	3.824	+0.534 $\pm 0.3798$ n = 5	0
Stroke volume ... cc/beat	62.50	+13.30 $\pm 7.979$ n = 4	0	64.50	+12.48 $\pm 5.389$ n = 5	0
Ventilation l/min.	6.672	+1.385 $\pm 0.3283$ n = 6	+20 %	6.672	+0.527 $\pm 0.2712$ n = 6	0
Resp. ....	0.866	+0.049 $\pm 0.0251$ n = 6	0	0.866	+0.053 $\pm 0.0109$ n = 6	0

1937, OSTERWALD and MEURER 1939, IAGNOV 1940). The preparation Deriphyllin (Homburg) was used in a dose of 1 cc, containing 0.2 gram of theophylline, and was given intravenously. Determinations were made 15—25 mins. and 40—50 mins. after the injection.

*Experiments on Normal Subjects.* The effect of theophylline diaethanolamine was studied in 5 normal subjects. The modified NIELSEN method with 600 cc depth of respiration was used. The results are shown in table 19. There were no significant changes in the arterio-venous difference, the cardiac output and the stroke volume. The oxygen consumption was moderately increased, both after 15—25 mins. and after 40—50 mins. There was also a moderate increase in the ventilation. This is not to be regarded as an

Table 20.

*Effect of theophylline diaethanolamine on the cardiac output in congestive heart failure. Dose 1 cc. Deriphyllin = 0.2 gram theophylline.*

Patient no.	Initials. Date of experiment	Before inj. I, II			III 15-25 min. after inj.				IV 40-50 min. after inj.			
		Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Cardiac output lit./Min.	Stroke Volume cc/beat.	Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Cardiac output lit./Min.	Stroke Volume cc/beat.	Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Cardiac output lit./Min.	Stroke Volume cc/beat.	Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Cardiac output lit./Min.
11	S. A. 9/5-41 ...	98.2 89.9	2.85 3.24	34.8 38.6	84.2	3.17	41.3	97.3	3.06	36.4		
11	S. A. 10/5-41 ...	97.2 91.1	2.76 2.90	32.8 34.5	88.7	3.09	36.1	89.0	3.12	35.9		
12	G. F. 2/8-41 ...	68.7 69.7	3.67 3.61	59.2 58.7	86.3	2.85	42.6	76.2	3.11	53.8		
13	T. B. 2/8-41 ...	104.0 —	2.21 —	36.8 —	92.4	2.61	46.7	101.6	2.18	47.7		
14	J. S. 22/10-41 ...	100.2 84.1	2.15 2.69	27.9 33.1	91.1	2.17	29.0	95.7	2.57	30.6		
15	J. F. 11/10-41 ..	91.7 104.0	2.89 2.62	47.6 42.5	86.9	3.16	48.3	96.3	2.92	45.7		
20	M. L. 12/2-42 ..	103.5 98.8	2.24 2.32	29.8 33.0	94.5	2.74	39.0	105.2	2.11	33.7		
25	J. W. 1/6-42 ...	91.4 86.6	2.67 2.76	52.1 48.0	95.1	2.95	46.2	90.3	2.84	48.3		
26	B. K. 22/10-42 ..	112.2 124.1	1.67 1.67	20.2 22.0	120.3	1.70	20.3	109.7	2.00	24.1		
27	S. Y. W. 20/10-42	86.8 96.6	2.32 2.18	58.7 57.1	97.6	2.36	58.7	100.0	2.31	58.4		

effect of respiratory stimulation, as there was no change in the respiratory quotient (the decrease after 40-50 mins. was not affirmed by analysis of variance,  $0.2 > P > 0.05$ ).

*Experiments on Patients with Congestive Heart Failure.* Ten experiments were made and the figures are given in table 20, the results in table 21. The action of the drug is similar to that in the normal subjects. No significant changes occurred in the arterio-venous oxygen difference, the cardiac output or the stroke volume, whereas there was a slight increase in the oxygen consumption and the ventilation, but no changes in the respiratory quotient. In the blood pressure no significant changes occurred. Thus no constant effect of theophylline on the cardiac output



Table 21.

*The effect of theophylline diaethanolamine in 10 cases of congestive heart failure. 10 experiments. 1 cc Deriphyllin = 0.20 gram theophylline was given intravenously.*

Functions	Mean of the averages of the initial values $\frac{I+II}{2}$	Mean of the values taken 15—25 mins. after the injection III	Mean of the differences $III - \frac{I+II}{2}$	Mean of the values taken 40—50 mins. after injection IV	Mean of the differences $IV - \frac{I+II}{2}$
Art.-ven. ....	95.33	94.01	— 1.33	96.13	+ 0.79
O <sub>2</sub> difference cc/l.			0		0
Change .....					
Oxygen consumption .....	237.0	253.6	+ 16.6 ± 5.31 + 7 %	257.4	+ 20.4 ± 3.92 + 9 %
Change .....					
Cardiac output ... l/min.	2.580	2.740	+ 0.160 ± 0.1179 0	2.715	+ 0.135 ± 0.0508 (+ 5 %)
Change .....					
Stroke volume.... cc.	40.24	40.85	+ 0.61	41.46	+ 1.22 ± 1.308
Change .....			0		0
Ventilation .....	7.885	8.651	+ 0.766 ± 0.1495	8.476	+ 0.591 ± 0.1639
l/min. n = 9			+ 10 %		+ 8 %
Change .....					
Resp. quotient.... n = 9	0.766	0.779	+ 0.012 ± 0.0183 0	0.737	— 0.029 ± 0.0137 0
Change .....					

could be proved, at least in the dose of 0.2 gram, though in some of the cases (nos. 11, 13, 15, 20) there were increases in the cardiac output amounting to about twice the standard deviation. At all events there can be no talk of an effect comparable to that of the heart glucosides.

The fact that theophylline in cases of heart insufficiency causes an increase in oxygen consumption without increasing the cardiac output also indicates that there is no direct action on the heart. It will be reasonable to presume the same for

all the xanthines, as these generally seem to differ from one another only in quantitative respects, theophylline being the most active. Their usage combined with heart glucosides seems suitable from this viewpoint.

## The Effect of Nicethamide and Metrazol on the Cardiac Output.

### Earlier Investigations.

#### Animal Experiments.

The effect of nicethamide and metrazol in insufficient heart lung preparations has been investigated by several authors (TRENDELENBURG 1929, GREMELS 1930, LEYKO 1930, GOLLWITZER-MEIER 1936). No conclusive effect on the heart has been observed by any of them. In animals with low blood pressure due to the anesthesia MÜLLER (1936) and GOLLWITZER-MEIER

Table 22.

*Effect of nicethamide on the cardiac output in congestive heart failure.  
Dose 2 cc (0.5 gram).*

Patient no.	Initials. Date of experiment	Before inj. I, II				III 30 min. after inj.				IV 60 min. after inj.			
		Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Art.-ven. output lit./Min.	Cardiac output cc/beat	Stroke Volume cc/beat	Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Art.-ven. output lit./Min.	Cardiac output cc/beat	Stroke Volume cc/beat	Art.-ven. O <sub>2</sub> -Diff. cc/lit.	Art.-ven. output lit./Min.	Cardiac output cc/beat	Stroke Volume cc/beat
11	S. A. 12/1-41...	106.6 103.6	2.47 2.57	30.8 31.4		102.6	2.57	29.2		114.9	2.24	26.1	
12	G. F. 27/8-41...	74.3 78.3	3.39 3.14	56.5 52.3		80.3	3.14	49.0		83.4	2.96	49.4	
13	T. B. 27/8-41...	94.7 101.1	2.52 2.55	49.4 50.0		101.2	2.59	48.9		95.6	2.76	51.1	
22	F. S. 15/1-42...	107.5 110.5	2.69 2.62	33.6 32.1		105.8	2.70	36.2		107.7	2.70	34.6	
23	M. K. 3/1-42...	129.0 151.7	1.71 1.56	14.3 14.1		135.5	1.73	15.0		136.7	1.61	14.0	
25	J. W. 22/1-42...	97.4 —	2.95 —	51.5 —		89.3	3.02	53.0		93.7	3.00	51.3	

Table 23.

*The effect of nicethamide in 6 cases of congestive heart failure. 2cc were given in intramuscular injection.*

Functions	Mean of the averages of the initial values $\frac{I+II}{2}$	Mean of the values taken 20—30 mins. after the injection III	Mean of the differences $III - \frac{I+II}{2}$	Mean of the values taken 50—60 mins. after the injection IV	Mean of the differences $IV - \frac{I+II}{2}$
Art.-ven..... O <sub>2</sub> difference cc/l.	104.35	102.45	- 1.90 ± 1.926	105.33	+ 0.98 ± 2.454
Oxygen consumption cc/min.	260.2	261.0	+ 0.8	260.8	+ 0.6
Cardiac output .... l/min.	2.597	2.625	+ 0.028 ± 0.0325	2.545	- 0.052 ± 0.0810
Stroke volume..... cc	38.97	38.55	- 0.42 ± 1.240	37.75	- 1.22 ± 1.239
Ventilation ..... l/min.	8.022	8.214	+ 0.192 ± 0.2522	8.174	+ 0.152 ± 0.2666
Resp. quotient ....	0.724	0.715	- 0.009	0.729	+ 0.005

(1936) found a rise in blood pressure and an increase in the cardiac output and stroke volume, which they ascribed to a stimulation of the vasomotor centre and increased venous blood supply.

#### Experiments in Normal Human Subjects.

HOEN and NEUTHARD (1937) made similar experiments with nicethamide and metrazol as those related above (p. 55) with xanthines, and found a considerable increase in the oxygen consumption, cardiac output and stroke volume. The same criticism applies here as before. LILJESTRAND and NYLIN (1941) did not observe any significant change either in the oxygen consumption, the cardiac output, the stroke volume, the heart rate or the blood pressure. Nor was there any sign of respiratory stimulation, as the respiratory quotient did not change.

Table 24.

The effect of Metrazol on the cardiac output in congestive heart failure.  
Dose 1 cc = 0.1 gram.

Patient no.	Initials. Date of experiment	Before inj. I, II				III 20-30 min. after inj.				IV 50-60 min. after inj.			
		O <sub>2</sub> -Diff. cc/lit.	Art.-ven. lit/Min.	Cardiac output cc/beat	Stroke Volume cc/beat	O <sub>2</sub> -Diff. cc/lit.	Art.-ven. lit/Min.	Cardiac output cc/beat	Stroke Volume cc/beat	O <sub>2</sub> -Diff. cc/lit.	Art.-ven. lit/Min.	Cardiac output cc/beat	Stroke Volume cc/beat
17	F. B. 11/11-41..	97.1 101.3	2.26 2.21	34.8 33.0		89.7	2.54	37.4		77.5	2.08		4.69
19	K. B. N. 2/1-42	130.1 133.4	2.36 2.38	— —		131.8	2.36	—		124.2	2.55	—	
26	B. K. 2/10-42..	105.4 114.1	2.09 2.00	28.0 25.0		124.8	1.86	26.5		130.4	1.81		22.0
27	S. V. 3/10-42 ..	82.0 92.2	2.83 2.51	71.1 61.7		91.9	2.64	58.4		93.8	2.58		57.8
28	J. H. 3/2-43 ...	96.0 93.5	2.51 2.58	42.9 41.1		95.5	2.09	31.5		88.9	—	—	
28	J. H. 4/2-43 ...	93.8 96.6	2.55 2.65	37.0 39.7		101.1	2.97	51.7		83.3	3.21		44.4
29	D. E. 12/2-43 ..	85.2 87.6	3.52 3.55	41.3 40.5		88.0	3.35	39.5		73.1	4.24		49.8

### The Author's Experiment.

*Nicethamide.* Six experiments were carried out on six patients with congestive heart failure. Two cc = 0.5 gram were given in intramuscular injection, and fresh determinations were made after 20-30 mins. and 50-60 mins. The figures of the arterio-venous oxygen difference, the cardiac output and the stroke volume are given in table 22, the results in table 23. No significant changes were observed, either in the circulation or in the respiration.

*Metrazol.* The effect was studied in 5 patients with congestive heart failure, tables 24 and 25. A dose of 0.1 gram was given in intramuscular injection and fresh determinations were carried out after 20-30 mins. and 50-60 mins. In these experiments, too, there were no significant changes to be found. The increase in the oxygen consumption after 1 hour (table 25) was not found to be significant by analysis of variance ( $P > 0.2$ ).

In case 19 the heart rate was so rapid and irregular, that had counting to be given up. The determinations on patients nos.

Table 25.

The effect of metrazol in 6 cases of congestive heart failure. I cc was given in intramuscular injection. I and II are the initial values, III the values taken  $\frac{1}{2}$  hour (20—30 mins.) after the injection, IV the values 1 hour (50—60 mins.) after.

Functions	Mean of the averages of the initial values $\frac{I+II}{2}$	Mean of the differences $III - \frac{I+II}{2}$	Change $\frac{1}{2}$ hour after the injection Percentage	Mean of the averages of the initial values $\frac{I+II}{2}$	Mean of the differences $IV - \frac{I+II}{2}$	Change 1 hour after the injection Percentage
Art.-ven. .... O <sub>2</sub> difference cc/l.	100.61 n = 7	+ 2.64 $\pm 2.79$ n = 7	0	100.61 n = 7	- 4.73 $\pm 5.333$ n = 7	0
Oxygen consumption ..... cc/min.	255.1 n = 7	+ 3.3 $\pm 10.78$ n = 7	0	257.2 n = 6	+ 10.3 $\pm 2.642$ n = 6	0
Cardiac output .. l/min.	2.579 n = 7	- 0.034 $\pm 0.1107$ n = 7	0	2.582 n = 6	+ 0.318 $\pm 0.1772$ n = 6	0
Stroke volume... n = 7	41.35 n = 7	- 0.52 $\pm 3.482$ n = 7	0	41.22 n = 6	+ 2.96 $\pm 4.090$ n = 6	0
Ventilation ..... l/min.	7.503 n = 7	+ 0.353 $\pm 0.6144$ n = 7	0	7.483 n = 7	+ 0.312 $\pm 0.1566$ n = 6	0
Resp. quotient .. n = 7	0.755 n = 7	+ 0.018 $\pm 0.026$ n = 7	0	0.755 n = 7	0.000 n = 7	0

28 and 29 were made with the latest modification of the NIELSEN method (600 cc depth of respiration), and these values may be regarded as absolute.

Patient no. 29 a case of Grave's disease with a basal metabolic rate of + 66 % showed a cardiac index of normal magnitude, 2.16 but the arterial-venous oxygen difference, 86 cc/l was higher than normal.

These results indicate that the analeptics mentioned have no direct effect on the heart, at least in the usual therapeutic doses, and there seems to be no reason for their employment in heart insufficiency. This naturally does not eliminate the possibility of a favourable action in cases with circulatory failure due to vasomotor paralysis.



out of the dead space, tending to give too high values of the arterio-venous oxygen difference. As constant values were obtained in double determinations, with a variation coefficient of 5.71 %, it was considered possible to establish changes in the cardiac output at one and the same time. The method in the form used, however, does not permit comparison of the values of cardiac output in various subjects or the study of the variations during the course of a decompensation. A modification of the method, assuring a sufficiently great depth of respiration to wash out the dead space, was developed during the investigation with the object of making it generally serviceable. In normal subjects good agreement was demonstrated between values obtained by this modification of the NIELSEN method and by the GROLLMAN method. In double determinations on healthy subjects a variation coefficient of 4.74 % was obtained.

Determinations of the oxygen consumption by the bag method according to DOUGLAS resulted in a variation coefficient of 2.56 %.

For the determination of the cardiac output the coefficient of variation was 5.84 %, of the stroke volume 7.05 %.

### The effect of Drugs on the Cardiac Output.

*Digitalis* administered intravenously in cases of congestive heart failure caused an immediate increase in the cardiac output and the stroke volume, combined with a decrease in the arterio-venous oxygen difference. The increase seemed to be maximal after  $1\frac{1}{2}$  hour and was still present after  $2\frac{1}{2}$  hours.

In normal subjects no significant changes occurred.

*Strophanthin* in cases of congestive heart failure had a similar effect which appeared more quickly and was of a shorter duration.

In normal subjects no significant changes were found.

*Theophylline*. No significant effect on the cardiac output could be proved, whereas a moderate increase in the oxygen consumption was found. This applied to both normal subjects and patients with congestive heart failure.

*Analeptics*. Metrazol and nicethamide in cases of congestive heart failure had no influence either on the circulation or on the respiration in the usual therapeutic doses.





## Data relating to the patients

V. O. C. = Vitium organicum cordis. A. I. = aortic insufficiency.  
 Degree of cyanosis, dyspnea, odema and lung congestion:  
 Degree of liver enlargement: + = liver palpable 1 finger breadth below th-

Subject No.	Date of admission Initials Sex Age	Diagnosis	Cyanosis	Dyspnea during rest.	Oedema	Weight loss	Liver enlargement	Venous pressure
The Grollms								
1	S. A. ♂ 42 years 16/2 39	V. O. C. incomp. + Endocarditis acuta et chron. (M. I. + M. S.). Died 23/3 39.	+	++	++	Increased in weight.	++++	35 cm
2	K. T. C. ♂ 44 years 11/3 39	V. O. C. incomp. (M. S. + A. I. + A. S.). Died 23/8. P. A. D.: En- docardit, chron. fibrocalculosa (M. S. + A. I. + A. S.) + Cirrhos. cardiaque hep.	+	-	+	2.3 kg.	+	16.5 cm
3	S. Y. W. ♂ 51 years 21/4 39	V. O. C. incomp. (A. I. + M. I. + M. S.).	+	+	+	2.0 kg.	+	16
4	S. R. ♀ 35 years 10/3	V. O. C. incomp. (M. I. + M. S.)	+	-	-	2.2 kg.	++	14
5	S. H. S. ♂ 53 years 12/3 39	Myodeneratio cor- dis incomp.	(+)	-	+	2.2 kg.	+	
6	O. J. ♀ 32 years 22/10 39	V. O. C. incomp. (M. I. + M. S.).	-	-	-	0.6 kg.	+	

26.

*with congestive heart failure.*

A. S. = aortic stenosis. M. I. = mitral insufficiency. M. S. = mitral stenosis.

light +; moderate ++, pronounced +++.

Costal arch. ++ = liver palpable 2 finger breadths below the arch etc.

Circulation time	Auscultation of the heart	X-ray of the heart Heart volume cc/m <sup>2</sup> body surface	X-ray signs of lung congestion	Electrocardiogram	Drug tests
ethod.					
30 sec.	Systolic murmur max. at apex. P <sub>II</sub> acc.	General enlargement 1100 cc/m <sup>2</sup>	++ Fluid in the right pleura	Right ventricular preponderance + Coronary insufficiency + Auricular fibrillation.	<sup>21</sup> / <sub>2</sub> Digitalis test. 1 cc Digiton i. v. had been given <sup>18</sup> / <sub>2</sub> and <sup>19</sup> / <sub>2</sub> .
42 sec.	Systolic and diastolic murmur, max. at apex.	1350 cc/m <sup>2</sup> . General enlargement, most pronounced in left heart.	++	Auricular fibrillation + Right ventricular preponderance + Coronary insufficiency.	<sup>18</sup> / <sub>3</sub> Digitalis.
5 sec. 1/3 39)	Systolic murmur, max. at apex. Diastolic murmur, max. in I <sub>III</sub> sin. and at the aortic valve. P <sub>II</sub> acc.	930 cc/m <sup>2</sup> . General enlargement, especially in the left ventr.	++ Small quantities of fluid in both pleurae.	Auric. fibrill. + Coronary insufficiency.	<sup>20</sup> / <sub>4</sub> Digitalis.
1 sec.	Presystolic, systolic and diastolic murmur max. at the apex. 1st. sound abrupt. P <sub>II</sub> acc.	Heart enlargement of the mitral type. 750 cc/m <sup>2</sup> .	++	Auric. fibrill. + Coronary insufficiency.	<sup>12</sup> / <sub>5</sub> Digitalis.
—	Systolic murmur, max. in I <sub>III</sub> sin, P <sub>II</sub> acc.	860 cc/m <sup>2</sup> . General enlargement.	++	Auric. fibrill. + Coronary insufficiency + Ventricular extrasystole.	<sup>20</sup> / <sub>5</sub> Digitalis.
sec.	Presystolic and systolic murmur at apex. Diastolic murmur in I <sub>II</sub> sin.	Heart enlargement of the mitral type 750 cc/m <sup>2</sup> .	++	Right ventricular preponderance + Coronary insufficiency. P-waves enlarged.	<sup>21</sup> / <sub>10</sub> Digitalis.

Sub- ject No.	Date of admission Initials Sex Age	Diagnosis	Cyanosis	Dysp- nea during rest.	Oedema	Weight loss	Liver en- largement	Venous pressure
The Nielsen								
7	A. W. ♀ 54 years 11/2 40	Cardio-arterio- nephrosclerosis + Hypertonia gra- vis + Incomp. cordis. Died 4/5.	+	+	+++	26.3 kg	++++	—
8	A. B. W. ♀ 51 years 30/1 41	V. O. C. incomp. (Congenital vi- tium ??)	++	++	+++	5.5 kg.	++++	19
9	E. L. ♀ 61 years 3/2 41	V. O. C. incomp. Died 12/10 42. P. A. D. Stenos. valv. tricusp. et pulmonal. (con- genital?)+Tumor hep.	++	+	+	5.6 kg.	? (Tumor hepatis)	24 cm
10	J. H. L. ♂ 65 years 22/2 41	Hypertonia + Myodegeneratio cordis incomp.	+	+	++	9.5 kg.	++	21 cm
11	S. A. ♂ 46 years 22/2 41	V. O. C. incomp. Died 22/1 41. P. A. D.: Aortitis (luct.?) + A. I. + M. I.	+	++	+	0.9 kg.	++	17 cm
12	G. F. ♂ 65 years 21/1 41	Cardiosclerosis c. Incomp. cordis	++	++	+++	17.0 kg.	+++	14 cm
13	T. B. ♂ 36 years 6/10 41	V. O. C. incomp. (M. I. + M. S.)	+++	+++	+++	3.4 kg.	+++ (Ascites)	28.5 cm

focus is slowly reabsorbing while also miliary tubercles are disseminating in the lungs, and increases the hilum lymph-adenitis; moreover joints and hematogenic outspreading in the spleen (Fig. 3) and in the liver. At the 6th week the caseation also in the hilum lymphnodes appears.

Successively the extent of the former miliary tubercles of the lungs increases, tending to conglomerate into granulomatous, mostly caseous foci and giving place to a diffuse proliferation of the specific granuloma and following thickening of the interalveolar septa. Concomitantly also the other tubercular affected organs, *i. e.* spleen and lymph nodes, show a diffuse granulomatous involvement of the interstitial connective tissue.

The caseous change of the granuloma in the lung reaches its maximum after 8 or 9 weeks during which the sclerotic transformation of the tubercular process begins everywhere in the affected organs. This tendency assumes a particular significance in the liver where the connective tissue proliferation develops giving an anatomical picture similar to that of liver cirrhosis, namely consisting on the degeneration of the parenchymatous cells, a diffuse increase of the connective tissue starting from the portal spaces and sometimes from the centrum of the lobuli; a considerable splenomegaly with the characters of a diffuse fibrous inflammatory reaction and partially also as a secondary effect to the cirrhosis and correlated state of the blood in the portal district, as usually happens also in the spontaneous cirrhosis of the liver. The only organs that showed little or no tendency to the sclerotic transformation were the tracheobronchial lymph nodes, sometimes the cervical ones, that were found caseous prevalently in the latest stage of the disease.

### Allergic Reaction.

The allergic reactivity evaluated, as above mentioned, on behalf both of the smallest sensibilizing tuberculin dose and from the superficial extent of the skin reaction by the constant anatuberculin test, showed a rather significative behaviour, desumed from the mostly uniform and linear results.

It was thus demonstrated that the *preallergic* period in the *subcutaneous* infected animals lasted about five weeks. Just at the end of the 5th week we could observe the first positive skin reaction, and successively a gradual slowly increasing reactivity

Sub- ject No.	Date of admission Initials Sex Age	Diagnosis	Cyanosis	Dysp- nea during rest.	Oedema	Weight loss	Liver en- largement	Venous pressure
14	J. S. ♀ 49 years 2/10 41	Hypertonia + In- comp. cordis + Intoxicatio di- gital.	+	+	—	1.3 kg.	++	16 cm (23/10)
15	J. F. ♂ 69 years 3/10 41	Cardiosclerosis c. incomp. cordis + Nephrosclerosis + Hypertonia.	++	+++	+++	19.8 kg	+++	—
16	G. H. ♂ 60 years 12/10 41	Hypertonia + Myodegeneratio cordis cum in- comp.	+	+	—	3.6 kg.	++	18.5 cm
17	F. B. ♂ 44 years 11/11 41	V. O. C. incomp. (A. I. + M. I.)	(+)	—	—	0.5 kg.	—	7 cm
18	A. S. ♀ 47 years 12/12 41	Hypertonia cum incomp. cordis.	(+)	—	+	8.6 kg. (1000 cal. diet) 15/12— 20/12 3.0 kg. weight loss.	++++	13 cm
19	K. B. N. ♂ 50 years 12/1 42	V. O. C. incomp. (M. I. + M. S.) + Myodegenera- tio cordis.	++	+++	+++	11.7 kg	+++ Ascites	18.5 cm
20	M. L. ♀ 69 years 12/2 42	Hypertonia + Myodegeneratio cordis incomp.	+	+	(+)	4.6 kg.	+	12 cm

Circulation time	Auscultation of the heart	X-ray of the heart Heart volume cc/m <sup>2</sup> body surface	X-ray signs of lung congestion	Electrocardiogram	Drug tests
26 sec. ( <sup>23</sup> / <sub>10</sub> )	Systolic murmur. Bigeminia.	(June 1941). General enlargement 830 cc/m <sup>2</sup>	++ (June 1941)	Auric. fibrill. + ventricular extrasystoles T <sub>I</sub> diphasic, T <sub>II</sub> and T <sub>III</sub> neg. S-T interval depressed Digitalis effects? <sup>15</sup> / <sub>10</sub> Auric. fibrill. + Coronary insufficiency. No extra systoles.	<sup>24</sup> / <sub>10</sub> Theophylline <sup>25</sup> / <sub>10</sub> Strophanthin <sup>28</sup> / <sub>10</sub> Digitalis.
—	Systolic murmur, max. at apex.	( <sup>21</sup> / <sub>10</sub> ). General enlargement. 1000 cc/m <sup>2</sup> .	++ Small quantity of fluid in the left pleura. ( <sup>21</sup> / <sub>10</sub> )	Auricular fibrillation + Left ventricular preponderance + Disturbances of the intraventricular conduction.	<sup>10</sup> / <sub>10</sub> Digitalis. <sup>14</sup> / <sub>10</sub> Theophylline
25 sec.	No murmur.	Not carried out.		Auric. fibrill. Heart rate 170/min. + Coronary insufficiency.	<sup>16</sup> / <sub>10</sub> Digitalis.
20 sec.	Systolic murmur, max. at apex. Diastolic murmur at the aortic valve.	General enlargement 800 cc/m <sup>2</sup> <sup>3</sup> / <sub>12</sub> 690 cc/m <sup>2</sup> .	++ Fluid in both pleurae. <sup>3</sup> / <sub>12</sub> no signs of lung congestion, no fluid in chest.	Coronary insufficiency + Disturbances of the intraventricular conduction	<sup>13</sup> / <sub>11</sub> Metrazol. <sup>14</sup> / <sub>11</sub> Strophanthin. <sup>17</sup> / <sub>11</sub> Digitalis.
—	Systolic murmur, A <sub>II</sub> and P <sub>II</sub> acc.	Enlargement most pronounced in the left ventricle. 590 cc/m <sup>2</sup> .	++	Left ventricular preponderance + Coronary insufficiency + Slight disturbances of the intraventricular conduction.	<sup>17</sup> / <sub>12</sub> Strophanthin <sup>18</sup> / <sub>12</sub> Digitalis.
47 sec.	Systolic and diastolic murmur, max. at apex.	Pronounced enlargement of the heart, mitral configuration. Measures cannot be given <sup>10</sup> / <sub>2</sub> Heart size considerably decreased 830 cc/m <sup>2</sup> . Fluid nearly vanished.	++ Fluid in the right pleura.	Auric. fibrill. Heart rate 155/min. Arrhythmisation block + Coronary insufficiency.	<sup>21</sup> / <sub>1</sub> Metrazol <sup>22</sup> / <sub>1</sub> Digitalis.
19 sec	Systolic murmur, max. at apex. A <sub>II</sub> acc.	Enlargement most pronounced in the left ventricle and atrium 870 cc/m <sup>2</sup> .	++	Left ventricular preponderance + Coronary insufficiency.	<sup>17</sup> / <sub>2</sub> Theophylline <sup>18</sup> / <sub>2</sub> Strophanthin.

Sub- ject No.	Date of admission Initials Sex Age	Diagnosis	Cyanosis	Dysp- nea during rest.	Oedema	Weight loss	Liver etc. Enlargement	Notes
21	J. S. ♂ 65 years 6/4 42	Hypertonia + Myodegeneratio cordis incomp.	+	+	+++	14.8 kg.		
22	F. S. ♂ 54 years 10/4 42	Cardiosclerosis cum incomp. cor- dis. Died 21/7 42. P. A. D.: Endo- carditis ulcerosa peracta cum ste- nos, et insuff. gravis valv. aor- tae.	+	++	—	4.3 kg.		
23	M. K. ♀ 39 years 2/5 42	V. O. C. incomp. (M. I. + M. S.)	++	+	++++	13.5 kg.		
24	V. W. ♂ 45 years 11/5 42	Cardioarterioscle- rosis cum in- comp. cordis.	+	++	—	5.0 kg.		
25	J. W. ♂ 64 years 26/5 42	Myodegeneratio cordis incomp.	+	(+)	—	2.6 kg.		
26	B. K. ♂ 46 years 10/10 42	V. O. C. incomp. (A. I. + M. I. + M. S.?)	+	+	+	4.3 kg.		
27 (3)	S. Y. W. ♂ 54 years 27/10 42	V. O. C. incomp. (A. I. + M. S. + M. I.) Died 20/11 42. P. A. D.: En- docardit. chron. valv. aortae cum insufficiencia + Embolia art. cerebri med. dxt.	+	++	+	1.4 kg.		
28	J. H. ♂ 42 years 1/2 43	V. O. C. incomp. (M. I. + M. S.)	+++	++	—	3.1 kg.		
29	D. E. ♀ 42 years	Thyreotoxicosis cum incomp. cor- dis.	—	(+)	—	12.3 kg. (after Lap- rocentesis of thoraco- centesis)		(A. R. 100)

Circulation time	Auscultation of the heart	X-ray of the heart Heart volume cc/m <sup>2</sup> body surface	X-ray signs of lung congestion	Electrocardiogram	Drug tests
55 sec.	Systolic murmur, max. at apex.	General enlargement 1070 cc/m <sup>2</sup> .	++	Auric. fibrill. + Coronary insufficiency.	<sup>10</sup> / <sub>1</sub> Strophanthin <sup>12</sup> / <sub>1</sub> Digitalis.
60 sec.	Systolic and diastolic murmur, max at apex.	General enlargement 1050 cc/m <sup>2</sup> .	++	Sinus tachycardia, rate 95/min. Left ventricular preponderance + Coronary insufficiency + Disturbances of the intraventricular conduction.	<sup>12</sup> / <sub>1</sub> Nicethamide <sup>14</sup> / <sub>1</sub> Strophanthin.
—	Systolic murmur, max. at apex. 1st sound abrupt and accentuated.	General enlargement most pronounced to the right and backwards. 1020 cc/m <sup>2</sup> .	++	Auric. fibrill. + single ventricular extrasystoles.	<sup>2</sup> / <sub>3</sub> Nicethamide <sup>7</sup> / <sub>3</sub> Strophanthin <sup>6</sup> / <sub>5</sub> Digitalis.
15 sec.	1st sound blurred.	General enlargement 870 cc/m <sup>2</sup>	++	Auric. fibrill + Coronary insufficiency.	<sup>12</sup> / <sub>1</sub> Strophanthin. <sup>12</sup> / <sub>5</sub> D.o.
45 sec.	Systolic murmur, max. at apex. P <sub>II</sub> accentuated.	General enlargement 1240 cc/m <sup>2</sup> .	++	Auricular fibrillation + Bundle-branch block.	<sup>1</sup> / <sub>6</sub> Deriphyllin <sup>2</sup> / <sub>6</sub> Strophanthin.
45 sec.	Systolic murmur, max. at apex. P <sub>II</sub> accentuated.	General enlargement 900 cc/m <sup>2</sup> .	++	Auricular fibrillation + Coronary insufficiency	<sup>21</sup> / <sub>10</sub> Metrazol <sup>22</sup> / <sub>10</sub> Deriphyllin.
43 sec.	No change since 1939. (see No. 3)	Configuration as 1939. 1280 cc/m <sup>2</sup> .	++	Auricular fibrillation + Left ventricular preponderance + Disturbances of the intraventricular conduction.	<sup>20</sup> / <sub>10</sub> Metrazol.
28 sec.	Systolic and diastolic murmur. P <sub>II</sub> accentuated.	General enlargement 800 cc/m <sup>2</sup> .	++ Fluid in both pleurae.	Auricular fibrillation + Right ventricular preponderance.	<sup>2</sup> / <sub>2</sub> Metrazol <sup>4</sup> / <sub>2</sub> Metrazol
16 sec.	Systolic murmur, max in I <sub>III</sub> sin.	Pronounced general enlargement, measures cannot be given.	(+) Moderate quantity of fluid in the right pleura.	Right ventricular preponderance + Disturbances of the intraventricular conduction.	<sup>12</sup> / <sub>2</sub> Metrazol.



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# ACTA MEDICA SCANDINAVICA

## Corrections

to Acta medica scandinavia vol. CXIII Supplementum CXLV.

*The Influence of Heart Glucosides, Theophylline and Analeptics on the Cardiac output in Congestive Heart Failure.*

On page 9, line 17, read adopted for adapted.

- 11, in the denominator of the formula read  $C_2H_2I \times \frac{N_2II}{N_2I}$  for  $C_2H_2 \times \frac{N_2II}{N_2I}$
- 16, line 8, read  $\sigma_t$  for  $\delta_t$
- 21, line 10, read a smaller coefficient of variation for less spreading
- 26, line 4 from the foot of the page, read Art. ven.  $O_2$ diff for Arte. vn.  $O_2$ diff.
- 30, line 4 from the foot of the page, read 5.33 for 5.23
- 33, Table 8, Difference I—II, read + 0.66 cc/l for - 0.66 cc/l.
- 41, line 1 from the foot of the page, read is due for are due
- 42, line 14, read Margolies for Margolier and Donal for Donat
- 45, line 18, read  $0.05 > P > 0.01$  for  $0.01 > P > 0.05$ .
- 47, line 5 from the foot of the page read  $0.05 > P > 0.01$  for  $0.05 < P < 0.01$
- 48, Table 15. Art.-ven.  $O_2$ difference Change 2½ hours after injection, read (- 7 %) for - 7 %
- 54, line 7 from the foot of the page, read nitrons for mitrons
- 56, Table 19, read Resp. quotient for Resp.
- 61, line 2 from the foot of the page, read counting had for had counting
- 71. case no 14, electrocardiogram, read  $T_I$  diphasic for  $T_I$  diphasic.

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# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CXLVI

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UNTERSUCHUNGEN ÜBER DEN  
EINFLUSS DER TONSILLITIS UND DER  
TONSILLEKTOMIE AUF DAS STERNAL-  
PUNKTAT UND DAS BLUTBILD

BEITRÄGE ZUR KENNTNIS DER GRANULOZYTOPENIE-  
ANGINA UND DER FOKALINFEKTION

VON

MIKKO VIRKKUNEN

# ACTA MEDICA SCANDINAVICA

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AUS DER MEDIZINISCHEN POLIKLINIK (VORSTAND PROF. DR.  
MED. ÖSTEN HOLSTI) UND DER OTO-LARYNGOLOGISCHEN KLINIK  
(VORSTAND PROF. DR. MED. YRJÖ MEURMAN)  
DER UNIVERSITÄT HELSINKI

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UNTERSUCHUNGEN ÜBER DEN  
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PUNKTAT UND DAS BLUTBILD

BEITRÄGE ZUR KENNTNIS DER GRANULOZYT-  
PENIE-ANGINA UND DER FOKALINFEKTION

VON

*MIKKO VIRKKUNEN*

HELSINKI 1943



HELSINKI 1943  
MERCATORIN KIRJAPAINO

## VORWORT.

Die vorliegende Arbeit wurde Anfang 1939 an der Medizinischen Poliklinik der Universität Helsinki in Angriff genommen. Die zuerst durch den finnisch-russischen Winterkrieg und dann durch den jetzigen Krieg geschaffenen aussergewöhnlichen Verhältnisse haben bewirkt, dass sich die Vollendung der Arbeit stark verzögert hat. Mein Material ist kleiner geblieben, als geplant war, da es mir seit dem Juni 1941 wegen des Militärdienstes nicht möglich gewesen ist, es weiter zu vermehren.

Indem ich diese Arbeit abschliesse, sage ich meinen herzlichsten Dank meinem hochverehrten Lehrer und früheren Chef, Herrn Professor Dr. med. ÖSTEN HOLSTI, der mir das Thema zu der Untersuchung gegeben, diese mit unermüdlichem Interesse verfolgt und mich, ohne Mühe und Zeit zu sparen, fortlaufend angeleitet hat.

Dem Chef der Oto-Laryngologischen Klinik, Herrn Professor Dr. med. YRJÖ MEURMAN, spreche ich meinen hochachtungsvollen Dank aus für seine wertvollen Ratschläge und für das Wohlwollen, das er meiner Arbeit entgegengebracht hat. Von der Oto-Laryngologischen Klinik habe ich Patienten für mein Material erhalten, und dort wurden auch an meinen Patienten die rhino-laryngologische Spezialuntersuchungen und die Tonsillektomie ausgeführt.

Dem Leiter des Städtischen Epidemiekrankenhauses in Helsinki, Herrn Dozent Dr. med. VILJO RANTASALO, danke ich ergebenst dafür, dass er mir in seiner Anstalt behandelte Fälle zur Verfügung gestellt hat.

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Wärmstens danke ich den Laborantinnen der Medizinischen Poliklinik der Universität, Fräulein TOINI VAINIO, LAILA ENSIO, HELMI HUTTUNEN und Frau Doktor MIRJAM KRUSIUS, für die Ausführung der Blutuntersuchungen.

Herrn Professor GUSTAV SCHMIDT, der mit grosser Sorgfalt die Übersetzung meiner Arbeit aus dem Finnischen in das Deutsche ausgeführt hat, sage ich besten Dank.

Die Direktion der EMIL AALTONEN-Stiftung hat für meine Untersuchung ein Stipendium bewilligt, das die Durchführung meiner Arbeit hochgradig erleichtert hat.

Helsinki, im April 1943.

Der Verfasser.

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## I. EINLEITUNG UND FRAGESTELLUNG.

Den Anstoss zu der vorliegenden Arbeit hat ein Fall (Nr. 21) gegeben, der im Mai 1938 an der Oto-Laryngologischen Klinik in Behandlung kam. In Frage stand eine an septischer Tonsillitis leidende Frau, die einer Tonsillektomie unterworfen werden sollte. Aus dem Blutbild ging jedoch hervor, dass die Patientin eine schwere Granulozytopenie-Angina hatte. Es wurde beschlossen (HOLSTI, MEURMAN), die Operation an ihr auszuführen, da zu vermuten stand, dass sie ohne sie ihre schwere Tonsilleninfektion und die daran anschliessende Granulozytopenie-Krise nicht würde überstehen können. Nach der Tonsillektomie erholte sich die Patientin schnell. Es zeigte sich, dass im Schrifttum über derartige Fälle nur wenig Angaben zu finden waren. Darum beschloss ich, die Möglichkeiten der Tonsillektomie bei den Granulozytopenie-Anginen genauer zu untersuchen. Es war mein Bestreben, mit Hilfe kasuistischer Fälle folgende Fragen näher aufzuklären:

1. *In welcher Weise wirkt eine im akuten Stadium ausgeführte Tonsillektomie klinisch und hämatologisch auf die Angina-Granulozytopenie-Krise, und*

2. *hat dieser Eingriff therapeutische Möglichkeiten?*

3. *Um die entsprechende Einwirkung auf die normal funktionierende Hämatopoese zu vergleichen, untersuchte ich das Sternalpunktat und das Blutbild vor und nach der Tonsillektomie in akuten Angina-fällen bei Personen, die keine Blutkrankheiten gehabt hatten.*

Ausserdem führte ich die erwähnten hämatologischen Untersuchungen bei Patienten mit chronischer Tonsillitis aus, um weiteres Licht über die mangelhaft bekannte Frage von der Einwirkung fokaler Entzündungsherde auf die Hämatopoese zu verbreiten. Durch meine Arbeit versuchte ich damit auch eine Antwort auf folgende Fragen zu finden:

4. Sind in den Sternalpunktionen und Blutbildern von Patienten mit chronischer Tonsillitis Abweichungen von der Norm festzustellen, und, bejahendenfalls, von welcher Art sind sie?

5. Gibt es eine unmittelbare Wirkung der Tonsillektomie auf das Sternalpunktat und das Blutbild bei diesen Patienten, und, bejahendenfalls, von welcher Art ist sie?

6. Sind nach der Tonsillektomie, wenn die mögliche direkte Wirkung der Operation schon vorüber ist, weitere Veränderungen in den Sternalpunktionen und Blutbildern zu finden, und, bejahendenfalls, von welcher Art sind diese?

Während der Arbeit stieg noch die Frage auf:

7. Können aus den Sternalpunktionen und Blutbildern Schlüsse auf die Funktionstüchtigkeit des Knochenmarkes bei Patienten mit chronischer Tonsillitis gezogen werden?

## II. METHODIK.

Die Wirkung der akuten Tonsillitis und der Tonsillektomie auf die Hämatopoese sowohl bei granulozytopenischen als bei gewöhnlichen Anginen wurde durch Verfolgung der Sternalpunktate und Blutbilder der Patienten untersucht. Auf die Frage, ob im Sternalmark und im Blutbild der an chronischer Tonsillitis leidenden Kranken Veränderungen aufzuzeigen sind, wurde in der Weise nach einer Antwort gesucht, dass die Punktate und Blutbilder dieser Patienten mit den bei Gesunden festgestellten Normalwerten verglichen wurden. Vergleicht man ferner die vor der Tonsillektomie sowie die zu verschiedenen Zeiten danach ausgeführten hämatologischen Untersuchungen und die Normalwerte miteinander, so ergibt sich auch die Möglichkeit, zu ermitteln, ob die Beseitigung des Tonsillenherdes den hämatologischen Status beeinflusst. Die allgemeine klinische Untersuchung der Patienten ist dabei von grosser Bedeutung, da wir mit ihrer Hilfe nachweisen können, ob sie ausser der chronischen Tonsillitis andere Krankheiten haben, die möglicherweise auf die Hämatopoese einwirken. Aus diesem Grunde habe ich alle Patienten sowohl vor der Tonsillektomie als bei den Nachuntersuchungen sorgfältig geprüft. Diese klinische Untersuchung wird später in anderem Zusammenhang genauer beschrieben.

Die Sternalpunktion wurde in Novocainanästhesie im allgemeinen unmittelbar nach der Blutuntersuchung oder in wenigen Fällen am Tage nach dieser ausgeführt. Bei dieser Anordnung vermeidet man eine Einwirkung des in dem Anästhetikum enthaltenen Adrenalins auf die Hämatopoese, was u. a. von BENHAMOU und NOUCHY sowie von PASCHIKIS und SCHWONER festgestellt worden ist. Knochenmarksmasse wurde bei der Punktion nur 0.2—0.3 cm<sup>3</sup> aspiriert, um einer grösseren Beimischung von Blut zu derselben vorzubeugen. Aus demselben Grunde tropfte



ich bei der Herstellung von Strichpräparaten auf das Objektglas zuerst einen grossen Tropfen aus der Aspirationsspritze und saugte den flüssigen Teil in die Spritze zurück, wobei auf dem Glase reichlich grauliche Knochenmarkspartikelchen blieben, die dann als Strichpräparat ausgebreitet wurden. Das Präparat wurde nach MAY-GRÜNWALD-GIEMSA gefärbt. Für die Bestimmung der Retikulozyten wurde auch eine Brilliantkresylblaufärbung ausgeführt. Bei der Differenzierung der Punktionspräparate teilte ich 400 Leukozyten ab und vermerkte zugleich, wieviel andere kernhaltige Zellformen auf diese Menge in dem Präparat vorhanden waren. Die Differenzierung dieser Zellmenge wird im allgemeinen als für die Gewinnung eines qualitativen Bildes von dem Sternalmark hinreichend angesehen (SEGERDAHL, TÖTTERMAN). Die Anwendung einer derartigen Differenzierungsweise empfiehlt ROHR, da sie in mancher Beziehung vorteilhafter ist als die direkte Angabe der Hundertsätze für die verschiedenen Zellarten des Sternalpunktates. Erstens ist bei diesem Verfahren ein Vergleich mit dem peripheren Blutbild leicht. Zweitens sind die Ergebnisse exakter. Die myeloischen Zellen im Sternalpunktat sind nämlich gleichmässiger über das ganze Strichpräparat verteilt, während die erythroischen und die Retikulumzellen oft in Gruppen liegen. Beim Zählen einiger hundert Zellen kann man daher oft im Durchschnitt entweder reichlich oder spärlich Erythroblasten bekommen, was nicht auf die Gesamtergebnisse einwirkt, weil die Zahl der Erythroblasten getrennt angegeben wird. In den Strichpräparaten ist eine Anhäufung der Zellen an den Rändern des Präparates zu beobachten. Aus diesem Grunde habe ich ungefähr gleich viel Zellen von den Rändern wie aus der Mitte des Präparates differenziert.

Bei der Einteilung der Knochenmarkszellen wurden dieselben Einteilungsprinzipien und Bezeichnungen wie u. a. von ROHR und TÖTTERMAN angewandt. Von einer genaueren Beschreibung der verschiedenen Zellen sehe ich in diesem Zusammenhang ab, aber ich berichte kurz über die Einteilungsprinzipien, die ich befolgt habe. Die Prototypen der Erythrozyten wurden in 3 Gruppen: in Proerythro-, Makro- und Normoblasten geteilt. Der jüngste Prototyp, der Proerythroblast, ist in dem Sternalpunktat selten, oft nur sporadisch anzutreffen. Die Unterscheidung der allerjüngsten Erythroblasten von den Myeloblasten bereitet oft Schwierigkeiten. Die Differenzierung wird dadurch erleichtert, dass um einen solchen Prototyp häufig ältere Erythrozyten auftreten, die eine kleine Zellgruppe mit ihm bilden. Die Makro- und Normoblasten wurden auf Grund der Grösse in der Art

in verschiedene Gruppen geteilt, dass als Normoblasten die kernhaltigen Erythroblasten galten, die die Abmessungen des gewöhnlichen Erythrozyten zeigen oder etwa doppelt so gross sind. Die noch grösseren Prototypen wurden als Makroblasten gezählt. Das Protoplasma der Normoblasten ist gewöhnlich polychromatisch oder oxyphil und das der Makroblasten basophil, aber mitunter stösst man auf Makroblasten, die ein oxyphiles Protoplasma aufweisen. Die Myeloblastengruppe bilden die jüngsten Prototypen der Leukozyten, deren Protoplasma stark basophil und deren Kernstruktur feinkörnig ist und Nukleolen enthält. Sobald im Protoplasma eine Granulation zu bemerken ist, handelt es sich schon um den Promyelozyten. Diese Zellgruppe umfasst auch verschiedene Reifestadien bis zu solchen myeloischen Zellen, deren Protoplasma oxyphil oder polychromatisch ist und die eine feine Granulation und einen verkleinerten, dichten und nukleolenfreien Kern zeigen. Derartige Zellen sind als reife Myelozyten gerechnet geworden, und zu derselben Gruppe gehören ferner die Zellen, deren Kerne nierenförmig, auf der einen Seite etwas eingezogen sind. Die folgende Gruppe der Metamyelozyten bilden reifere Zellen bis zu solchen, deren Kern schmal, hufeisenförmig ist, wobei es sich schon um Stabkernige handelt. Zu den Stabkernigen sind auch die Zellen gezählt worden, in denen sich Anfänge einer Segmentation des Kernes feststellen lassen, obgleich zwischen den verschiedenen Segmenten keine fadenförmigen Verbindungsteile zu sehen sind. Zu der Gruppe der Retikulum- und Plasmazellen sind die Plasmazellen, die selten vorkommenden phagozytierenden Zellen sowie die sogenannten grossen und kleinen lymphoiden Retikulumzellen gestellt. Die grossen lymphoiden Retikulumzellen sind verhältnismässig leicht durch das lockere, alveolare Chromatinnetzwerk und ihr schwach basophiles, oft azurophile Granula enthaltendes Protoplasma von den anderen Zellgruppen zu sondern. Dagegen kann die Unterscheidung der den ersteren ähnlichen kleinen lymphoiden Retikulumzellen von den gewöhnlichen Lymphozyten Schwierigkeiten bereiten. Da in mehreren meiner Fälle im Knochenmark eine beträchtliche Lymphozytose auftritt, will ich hervorheben, dass ich diesem Verhalten Beachtung geschenkt habe.

Die Sternalpunktion ist eine junge Untersuchungsmethode, aber ihre Vorteile und Grenzen sind bekannt. Sie ist eine Stichprobe, mit deren Hilfe wir Aufschlüsse über den Zustand des Knochenmarkes im Untersuchungsmoment erhalten. SEGERDAHL hat gezeigt, dass, wenn man aus den Punktaten ein und desselben Individuums die Menge der Zellen je 1 mm<sup>3</sup> berechnet, die Ergebnisse erheblich schwanken. Zu den Verschiedenheiten trägt vor allem bei, dass sich dem Punktat immer bei der Aspiration mehr oder weniger Blut beimischt. In einem Ausnahmefall kann die Punktationsnadel ein Blutgefäss des Markes treffen, so dass man beim Aspirieren ausschliesslich Blut bekommt. In meinen eigenen Punktaten habe

gluconeogenesis, however, decreases after extirpation of the pituitary, while after injection of adrenal cortex hormone in hypophysectomized animals the gluconeogenesis increases. For this reason investigations were made to discover if the effect of the anterior pituitary on carbohydrate metabolism was conveyed by way of the adrenal cortex.

Viale's experiments (25) were of special interest in this connection. He pointed out that glucosuria after total or partial pancreatectomy on dogs decreased when the adrenals were extirpated. Epinephrectomy thus had a favourable effect on pancreatogenic diabetes similar to that of hypophysectomy. This effect is only obtained after removal of the adrenal cortex, not after demedullation or denervation of the adrenals. By administration of adrenal cortex hormone to pancreatectomized and epinephrectomized animals it was possible to produce hyperglycemia and glycosuria, and on large doses the values of the urine sugar excreted in 24 hours might exceed those found in pancreatectomized animals before the epinephrectomy. The adrenal cortex hormones thus have a diabetogenic affect.

Recent investigations have proved that the diabetogenic anterior pituitary hormone has to act together with the adrenal cortex to produce its full effect on carbohydrate metabolism. (Lundberg (26).) Injection of the diabetogenic anterior pituitary hormone has no effect on the blood sugar level, and there is no increase of the glycogen reserves of the liver in animals in which epinephrectomy has been performed. However, the glycogen content in the muscles rises. Thus it seems as if the regulation of the muscle glycogen is directly connected with the anterior pituitary, while the liver glycogen and the blood sugar are regulated by way of the adrenal cortex.

In pancreatectomized and epinephrectomized rats the anterior pituitary hormone, which was fully active in animals lacking the pituitary and the pancreas, did not cause glucosuria. If these animals, however, were given a certain amount of adrenal cortex hormone, there occurred abundant glucosuria after injection of ant. pituitary extract. From this Russel and Long (citation: Christensen (20)) came to the conclusion that a certain amount of adrenal cortex hormone was necessary to enable the pituitary factor to have any effect. It was evident that there is a synergism between the diabetogenic ant. pituitary hormone and the adrenal cortex hormones. (Long & Katzin (27) & Fry (28).)

korrigierten Hämoglobinwerte zur Verwendung gekommen. Am Epidemiekrankenhaus war die Korrektur (1,17) eine andere als an der Medizinischen Poliklinik. Infolgedessen sind die an den verschiedenen Krankenhäusern vorgenommenen Hämoglobinbestimmungen nicht ganz miteinander vergleichbar. In bezug auf die Differenzierung der Leukozyten besteht die Möglichkeit, dass die Prinzipien der Einteilung der Neutrophilen in Segmentkernige, Stabkernige und Metamyelozyten in den verschiedenen Laboratorien einigermassen variieren, und das kann die Zuverlässigkeit der Ergebnisse beeinträchtigen. Die von diesen kleinen Verschiedenheiten der Methoden herrührenden Nachteile betreffen jedoch nur 12 Patienten, nämlich die Granulozytopenie-Patienten und die akuten Anginafälle mit gewöhnlicher Blutreaktion. Für die Ergebnisse besitzt es keine praktische Bedeutung, dass die Blutbilder dieser Patienten nicht in demselben Laboratorium ausgeführt sind, da die Abweichungen des Blutbildes von der Norm bei ihnen gross waren.

Die Blutbilder der Normalfälle und der Tonsillitis chronica-Patienten wurden nach der gleichen Technik an der Medizinischen Poliklinik und der klinischen Abteilung ausgeführt. Bei der Bestimmung des Hämoglobins wurden da immer dieselben drei Sahli-Apparate benutzt. Die Mittelwerte der wiederholten Parallelbestimmungen, die in demselben Blute mit verschiedenen Hämometern von allen Laboratoriumsschwestern (4 Personen) vorgenommen wurden, unterschieden sich nur um 1 v. H. voneinander. Bei der Bestimmung des korrigierten Hämoglobinwertes konnte also für alle Schwestern und Hämometer die gleiche Korrektur angewandt werden. Für die Ermittlung der Korrektur wurden in demselben (Oxalat-) Blut die Hämoglobinbestimmungen einerseits mit den erwähnten Apparaten und andererseits im Mariakrankenhaus mit einem schon früher im Zoophysiologicalen Institut zu Kopenhagen standardisierten Apparat ausgeführt (wobei sie von einer Person gemacht wurden, deren Korrektur früher berechnet worden war). Nach der letzteren Bestimmung war der korrigierte Hämoglobinwert der Probe 97.7 %, was dem bei der ersterwähnten Bestimmung erhaltenen (unkorrigierten) Wert 90 % entsprach. Hiernach ist die Korrektur 1.08. Die Hämoglobinwerte sind sowohl unkorrigiert als korrigiert angeführt. Die Verdünnungsgläser wurden nach früher standardisierten Gläsern kalibriert. Bei



### III. MATERIAL.

Mein Untersuchungsmaterial umfasst im ganzen 77 Personen. Von diesen waren 20 subjektiv und objektiv gesunde Normalfälle, 6 Patienten litten an Granulozytopenie-Angina, 6 hatten eine gewöhnliche Tonsillitis im akuten und 45 eine solche im chronischen Stadium.

#### A. Die Normalfälle.

Die Ergebnisse der morphologischen Untersuchung des Blutbildes und des Sternalpunktates können nicht völlig zuverlässig mit den Resultaten anderer Forscher verglichen werden, weil sowohl die subjektiven Einteilungsgründe als die Technik der verschiedenen Autoren stets etwas voneinander abweichen. Das gilt für das Blutbild ebenso wie vor allem für das Sternalpunktat, bei dem die Einteilungsprinzipien bezüglich der verschiedenen Zellgruppen hochgradig variieren können. Jeder Forscher muss daher notwendigerweise gesunde Individuen untersuchen, um von dem normalen Knochenmark und dem Blut ein Bild zu gewinnen, mit dem er seine bei verschiedenartigen Krankheitszuständen erreichten Untersuchungsergebnisse vergleichen kann. Das Normalmaterial mancher Forscher (z. B. FORSELL, NORDENSON, TÖTTERMAN) enthält u. a. Personen, die nicht gesund waren, sondern bei denen Krankheitszustände vorlagen, die bekanntermassen nicht auf die Hämatopoese einwirken, wie Neurosen und Geisteskrankheiten. Bei der Einsammlung der Normalfälle habe ich zusammen 27 Personen untersucht. Da sich bei einem Teil von ihnen in der Anamnese oder im Status wiewohl unbedeutende auf eine Infektion hinweisende Umstände fanden, habe ich nicht diese ganze Zahl verwertet, sondern mein Material umfasst 10 Männer und 10 Frauen. Diese Personen waren gesund bis auf eine, die an epileptiformen Anfällen gelitten hatte. Die Frauen waren hauptsächlich Kontoristinnen und

Tabelle 1.  
Klinische Angaben über die Normalfälle.

Nr.	Alter	Ge- schlecht	Beruf	Anamnese	Allgemein- zustand	Tonsillenstatus	Zahnstatus
1	24	♀	Studentin	Als Kind Masern, sonst ge- sund gewesen.	O. B.	O. B.	Alle Zähne erhal- ten. 5 plombiert. Keine Wurzel- füllungen.
2	28	♀	Magister	Als Kind Masern und Schar- lach ohne Komplikationen.	O. B.	O. B.	Keine kariösen Zähne, zahl- reiche Plomben.
3	34	♀	Kinderheim- vorsteherin	Als Kind oft Rachenschmer- zen, weshalb mit 8 Jahren Enukleation der Tonsillen. Danach gesund.	O. B.	Tonsillen fehlen.	2 Zähne fehlen. Zahlreiche Plom- ben. Keine Wur- zelfüllungen.
4	29	♀	Kontoristin	Als Kind Masern und Stiek- husten. Während der Schulzeit Neigung zu Schnupfen und Husten. Später gesund.	In beiden Lap- pen der Gl. thy- reoidea ein pfau- mengrosses Ade- nom. Sonst o. B.	O. B.	O. B.
5	21	♀	Kontoristin	Hatte vor 3 J. Angina. Sonst gesund gewesen.	O. B.	O. B.	2 Zähne fehlen. Zahlreiche Plom- ben. Keine Wur- zelfüllungen.
6	22	♀	Kontoristin	Immer gesund gewesen.	Beide Lappen d. Gl. thyreoidea pfau- mengross, klein- knotig. Sonst o. B.	O. B.	8 Zähne fehlen, an 4 oberfläch- liche Karies.
7	26	♀	Typographin	Vor 3 Mon. »Influenza«, wobei eine Adnexitis, die nach 2 Wochen geheilt war. Sonst gesund gewesen.	O. B.	O. B.	An mehreren Zähnen ober- flächliche Karies.
8	28	♀	Schaffersfrau	Immer gesund gewesen.	O. B.	O. B.	An 2 Zähnen Karies.

O. B.

Keine Karies, keine  
Zahnfüllungen.

O. B.

Keine Karies, keine  
Zahnfüllungen.

O. B.

Keine Karies, keine  
Zahnfüllungen.

O. B.

N	10	11	Landwirts- tochter	K	Kommt zur Untersuchung, weil ihre Beine beim Stehen und Gehen müde werden und sie Schmerzen in den Fuss- sohlen bekommt. Sonst gesund gewesen.	Im r. Lappen der Gl. thyreoiden ein daumenspit- zengrosses Ade- nom. Pes planus l. n.	Daumenspitzen- gross, grubig. Kein Sekret.	O. B.
10	20	♀	Kontoristin		Hat als Kind Masern und vor 5 J. Angina gehabt.	O. B.	O. B.	Einige Plomben. Keine Wurzelfül- lungen.
11	21	♂	Unterfeld- weibel		Hatte als Kind 2 mal Angina. Vor 3 J. im akuten Stadium Tonsillektomie.	O. B.	Tonsillen fehlen.	O. B.
12	20	♂	Unterfeld- weibel		Als Kind Mittellohrentzündung ohne Komplikationen. Vor 1 J. Leistenbruchoperation.	O. B.	O. B.	O. B.
13	20	♂	Soldat		Immer gesund gewesen.	O. B.	O. B.	1 kariöse Wurzel.
14	20	♂	Korporal		Immer gesund gewesen.	O. B.	O. B.	An 3 Zähnen oberflächliche Karies.
15	20	♂	Soldat		Immer gesund gewesen.	O. B.	O. B.	O. B.
16	20	♂	Korporal		Als Kind Neigung zu Schnup- fen. Vor dem 10. J. Tonsillo- tomie.	O. B.	Auf beiden Sei- ten nur etwas Tonsillengewebe vorhanden.	O. B.
17	25	♂	Unterfeld- weibel		Immer gesund gewesen, ausser rechl. Karies. Vor 2 J. Zahn- sanierung.	O. B.	O. B.	Prothesen. Un- ten nur noch 2 Zähne.
18	23	♂	Unterfeld- weibel		Immer gesund gewesen.	O. B.	O. B.	O. B.
19	17	♂	Schüler		Immer gesund gewesen.	O. B.	O. B.	O. B.
20	30	♂	Lagerarbeiter		4 J. lang epileptiforme Anfälle mit 1—2 monatigen Intervallen. Letzter Anfall vor einer Woche. Hat keine Medika- mente genommen.	O. B.	O. B.	O. B.



Tabelle 2.

Das normale Sternalpunktat<sup>1</sup>.

Das normale Sternalpunktat *																			
Nr.	%										Zahl auf 400 L							Retikuloz.	%
	Bas. Myeloz. & Leukoz.	Eos. Myeloz.	Eos. Leukoz.	Myelobl.	Promyelo.	Myelo.	Metamylo.	Neutr. stabk. Leukoz.	Neutr. segm. Leukoz.	Lymphoz.	Mono.	Retikulum- & Plasmaz.	Megakar.	Normobl.	Makrobl.	Proerythrobl.	Rote Mitosen		
1	0.25	8.75	3.00	0.50	4.25	14.50	17.50	8.50	23.50	18.25	1.00	13	1	64	2	0	2	0	0.8
2	0	0.75	0.50	0.50	5.00	14.50	17.75	15.50	31.00	16.25	0.25	3	0	50	2	0	0	0	1.1
3	0.25	1.00	1.25	0.75	2.25	8.75	10.50	8.25	42.75	23.00	1.25	8	0	23	1	0	1	0	0.3
4	0	3.50	0.25	0.75	4.00	15.50	19.25	16.75	25.00	13.50	1.50	7	0	44	0	0	3	1	1.1
5	0	4.25	0.50	1.00	5.25	13.50	21.25	13.50	25.50	12.50	2.00	7	0	58	4	0	4	0	1.6
6	1.00	2.50	1.00	0.75	4.75	10.25	10.25	10.75	31.75	25.00	1.00	4	0	39	3	2	1	0	1.8
7	0.25	0.25	1.00	1.25	5.25	8.50	15.00	9.50	32.00	24.00	3.00	4	0	81	5	0	0	0	0.7
8	0.25	6.00	2.25	1.00	6.50	15.75	19.75	12.50	24.50	11.25	0.25	13	0	38	7	0	1	2	1.5
9	0.50	3.50	1.25	3.00	7.50	16.75	19.50	12.75	23.50	11.50	0.25	9	0	61	7	1	3	2	1.5
10	0.50	0.50	1.00	1.75	2.50	13.00	15.75	9.00	34.50	19.25	2.25	6	0	37	4	0	1	0	0.6
11	0	1.25	0	1.25	5.50	15.00	22.25	19.00	23.75	11.25	0.75	7	0	58	3	1	2	0	2.4
12	0	1.25	0.25	0.75	2.25	15.50	19.25	15.25	36.25	10.25	0.25	10	1	35	2	0	1	0	1.1
13	0	5.00	0.25	1.25	6.75	17.50	21.00	12.25	23.00	11.75	1.25	7	0	101	5	1	2	1	0.4
14	0.25	4.25	0.50	1.00	5.75	18.50	20.00	12.00	26.25	9.75	1.25	7	0	54	3	0	1	0	1.0
15	0.50	1.00	0.75	1.00	4.50	17.50	19.00	12.75	29.00	12.50	1.50	12	1	63	3	0	2	0	1.3
16	0.25	4.25	0.50	2.25	3.00	21.50	20.25	13.00	24.25	9.50	1.25	9	0	106	3	1	2	0	1.6
17	0	2.00	0.75	0.75	3.25	14.50	24.50	17.00	28.00	7.75	1.50	16	0	70	3	1	2	0	2.4
18	0.75	2.75	1.25	1.25	3.00	15.25	17.50	8.75	32.25	14.50	2.50	5	1	74	4	0	1	0	0.9
19	0.50	5.25	0.75	2.00	9.25	17.50	20.25	14.25	21.75	7.75	0.75	9	0	111	10	0	1	3	1.1
20	0.75	3.50	3.00	1.50	4.50	11.50	18.50	11.75	31.75	12.00	1.50	5	0	65	7	0	1	1	0.8

Dezimalen gebraucht, obwohl  
üblich, wie Blutbilder, werden der Sternalpunkte und  
in den Werten der Sternalpunkte vorausgesetzt.

Retikuloz. Dezimalen gebraucht, obwohl  
 1 In vorliegender Arbeit sind in den Werten der Sternalpunkte und Blutbilder, wie üblich, Dezimalen gebraucht, obwohl die Ungenauigkeit d

diabetes. Maranon & Morros (38) found among 115 cases of dystrophia adiposo-genitalis following hypofunction of the pituitary only 2 cases of diabetes mellitus. But hypofunction of the anterior pituitary does not always exclude diabetes mellitus. (John (67), Allan & Rowntree (68), Feldman, Roberts, Susselman & Lipetz (69).)

## II.

### The Effect of Pituitary on Basal Metabolism.

The pituitary is superior to the thyroid and governs its activity by aid of the thyreotropic hormone (Aron (70), Loeb et al. (71)). After hypophysectomy regressive changes appear in the thyroid. The thyroid has also an effect on the vegetative centres of the mid-brain and other endocrine organs, and is closely in tune with them. This mutual dependence is necessary for an orderly metabolism. The thyreotropic hormone in the anterior pituitary stimulates the hormonal secretion of the thyroid. This effect could successfully be demonstrated in young guinea-pigs. After injection of anterior pituitary extract the cells of the thyroid follicles swell out, the vesicles become smaller, the colloid is vacuolised, and proliferative changes in the cells take place. The result is increased secretion of thyroid hormone in the blood and a higher basal metabolic rate. This reaction is so characteristic that it is used as a test for the thyreotropic hormone.

The thyreotropic hormone has been isolated from the other pituitary hormones and has been found in watery extracts from the anterior pituitary. After injection of the hormone, besides an increase of the basal metabolic rate, also exophthalmus, tachycardia and increased irritability can be observed, and the thyroid gland increases in size, while the iodine content diminishes. In thyreoidectomized animals no such symptoms are seen, which proves that the effect occurs by way of the thyroid gland. If the hormone acts for a longer time, the stimulating effect on the thyroid is diminished, which should be due to the so-called anti-hormones with antagonistic effect on the thyreotropic hormone. But many authors deny the existence of these antihormones.

After hypophysectomy the basal metabolic rate falls, and adjusts itself after a time to a new and lower level. But it is some dissension between the various authors how much it falls. Means (72) states that the thyroid still functions after hypophysectomy,

Tabelle 3.

Variationsgrenzen und Mittelwerte des normalen Sternalpunktates.

	Rohr	Nordenson	Forssell	Tötterman	Virkkunen
Bas. Myeloz. & Leukoz.	0.10—1.0 0.38	0—1.25 0.13	0—0.42 0.16	0—0.50 0.15	0—1.0 0.3
Eos. Myeloz.	1.80—6.0	0—6.25 2.22	0.75—2.83 1.94	0.75—7.50 2.3	0.25—8.75 3.08
Eos. Leukoz.	4.0	0.25—7.50 1.84	1.17—3.42 2.08	0.50—8.50 1.75	0—3.0 1.0
Myelobl.	0.60—2.30 1.0	0.25—5.50 2.67	0.33—2.0 0.96	0.25—3.0 1.1	0.50—3.0 1.23
Neutr. Promyeloz.	4.50—15.10 10.0	1.25—8.25 4.02	0.83—3.17 1.86	0.50—8.50 3.7	2.25—9.25 4.75
Neutr. Myeloz.	3.30—11.70 7.0	4.25—18.0 9.25	9.83—18.67 13.37	5.0—21.75 15.7	8.50—21.50 14.76
Neutr. Metamyeloz.	4.10—10.30 7.0	9.25—42.50 28.36	27.25—38.42 32.38	3.75—28.0 16.3	10.25—24.5 18.44
Neutr. stabk. Leukoz.	48.20—65.0 41.0	2.0—10.75 5.66	4.25—9.50 6.73	5.25—19.25 12.16	8.25—19.0 12.65
Neutr. segm. Leukoz.	11.40—29.20 17.0	14.25—35.0 21.2	16.67—28.33 22.39	22.0—47.25 29.3	21.75—42.75 28.5
Lymphoz.	4.90—18.0 11.0	7.50—38.0 19.98	11.25—20.67 15.78	5.25—28.25 15.6	7.50—25.0 14.06
Monozy.	0.60—2.80 2.0	0—5.0 1.22	0.83—3.17 2.11	0—5.0 2	0.25—3.0 1.26
Retikulum- & Plasmaz.	1.6—16.8 100 L 8.0:100 L	0—3.75 400 L 1.56:400 L	2—6.83 400 L 4.63:400 L	0—11 400 L 2.6:400 L	3—16 400 L 8.0:400 L
Ferrataz.	—	3—40 400 L 15.72:400 L	—	0—7 400 L 0.95:400 L	—
Megakar.	—	0—1 400 L 0.11:400 L	0—0.67 400 L 0.27:400 L	0—2 400 L 0.27:400 L	0—1 400 L 0.2:400 L
Normobl.	13.9—43 100 L	26—138 400 L 83.94:400 L	29.01—117.67 400 L	15—138 400 L 77.4:400 L	23—111 400 L 61.6:400 L
Makrobl.	30:100 L	1—20 400 L 4.25:400 L	70.39:400 L	0—17 400 L 3:400 L	0—10 400 L 3.8:400 L
Proerythrobl.	0.42:100 L	—	0.98:400 L	1.45:400 L	1.55:400 L
Rote Mitosen	0.25:100 L	—	0.3—1.9 0.85	0.23:400 L	0.5:400 L
Weisse Mitosen	1.5—2.1	—	—	0.3—2.3 0.85	0.3—2.4 1.2
Retikuloz. %	—	—	—	—	—

of the thyreotropic anterior pituitary hormone. Attempts have been made with injections of the hormone into patients suffering from hypothyroidism, but the results have not been favourable. No increase of the secretion of the thyreoid can be produced by injection of thyreotropic hormone in these patients. The explanation is probably that the changes in the thyreoid gland in myxedema are so marked that the gland is in great part converted into fibrous tissue, and on fibrous scar tissue no parenchyma will grow. (Nielsen (77).) Moreover, the usual finding in myxedema is an increased content of thyreotropic hormone in the blood. This fact speaks against the theory of insufficient secretion of thyreotropic hormone as the cause of ordinary myxedema in man. Neither have there been found any signs of pituitary diseases on post mortem examination of cases with myxedema. Most often the pituitary is completely normal. In some cases small basophile adenomas have been found, but it is not proved that they are of any pathological significance for the hypothyroidism.

But still it is supposed that in certain cases diseases of the pituitary are able to cause hypothyreoidal conditions. A number of publications respecting myxedema in patients suffering from hypophyseal tumors have been issued (Leth Pedersen (78)). Transitional myxedema after trauma capitis (Jensen (79)) may also be the result of functional disturbances of the pituitary.

Snapper, Hunter et al. (80) have described a peculiar syndrome which they explain as a separate nosological unit, caused by pituitary disease. Characteristic for the syndrome is reduced basal metabolic rate, achylia, anemia, acrinia, hypogonadism and myelitic changes. They also saw excellent effects of a preparation containing thyreotropic anterior pituitary hormone. Some other authors, however, have denied that this syndrome has any connection with pituitary complaints and interpret it as being a peculiar form of spontaneous myxedema in adults, accompanied by anemia and assuming a pernicious character. The reason for the anterior pituitary extract having an effect should be its content of a certain amount of thyroxin. (Nielsen (77).) The question is not, however, settled, and the problems respecting the hypophyseal myxedema are still open to discussion.

The case which is to be discussed in the following pages will be of great interest as an illustration of the problems as to the pathogenesis of the diabetes mellitus and the hypothyreodism in man.



der Basophilen, Eosinophilen und Monozyten sind auch keine erheblichen Unterschiede zwischen den Werten SCHILLING's und anderer zu erkennen. Dagegen ist eine Differenz in den Prozentzahlen der Neutrophilen und Lymphozyten feststellbar. Die Variationsgrenzen der Neutrophilen (sowohl der Stab- als der Polymorphkernigen) sind bei den Finnen 47—71 und ihr Mittelwert 60. Die entsprechenden Zahlen SCHILLING's sind 54—72 und 67. Die Variationsgrenzen und der Mittelwert der Lymphozyten sind bei den Finnen 21—40 und 31 und bei SCHILLING 21—35 und 23. Auch bei Berücksichtigung der absoluten Zahlen sind die Lymphozytenwerte bei den finnischen Forschern durchgängig etwas höher als bei SCHILLING. Der höchste mittlere absolute Lymphozytenwert (1924) findet sich bei mir und der nächsthöchste (1869) bei APPELBERG.

Über die normale Menge der Blutplättchen besteht vorläufig keine volle Klarheit. Die nach verschiedenen Berechnungsmethoden erhaltenen Werte sind schwankend. Mit dem Verfahren von FONIO, das in der vorliegenden Arbeit angewandt ist, findet man als normale Menge der Thrombozyten 250000—300000. In meinen eigenen Fällen beträgt der Mittelwert der Thrombozyten 267980.

Die Senkungsreaktion war bei den Männern durchschnittlich 4 mm/St mit den Variationsgrenzen 1—10. Bei den Frauen lauteten die entsprechenden Zahlen 8 mm/St und 3—10. Bei den Männern waren die Werte in 7 Fällen 5 mm/St oder darunter und bei den Frauen entsprechend in 5 Fällen 8 mm/St oder weniger. Die obere Grenze der normalen Senkungsreaktion lässt sich nicht genau ziehen. Die Grenzwerte WESTERGREN's sind für Männer 7 mm/St und für Frauen 11 mm/St. FÄHRAEUS, LEFFKOWITZ, LINDSTEDT und REICHEL rechnen bei Männern noch 10 mm/St und bei Frauen 12—13 mm/St als normal. Meine eigenen Ergebnisse entsprechen also den im Schrifttum angeführten Normalwerten.

## B. Die Fälle mit Angina granulocytopenica.

### 1. Über die Granulozytopenie mit besonderer Berücksichtigung der Beziehung zwischen Tonsillitis und Granulozytopenie.

Den Anstoss zu der stark angeschwollenen Forschung über die Granulozytopenie-Krise gaben die 1922 von W. SCHULTZ mit-

Tabelle  
Variationsgrenzen und Mittel.

Autor	Zahl der Fälle	Ge- schlecht	Hb	Erythrozyt.	Index	Leukoz.
Schilling		♂ <sup>1</sup>				5000—8000 6000
Lindström u. Tallqvist	35	♂	95—105 98	4800—5320 5049	0.91—1.05 0.98	4500—6800 5549
	35	♀	80—90 87	4410—4690 4537	0.90—1.02 0.96	4100—8000 5683
Sandelin	2	♂				4800—6340 5570
	9	♀				4670—7280 5874
Becker	22	♂	83—107 93	4940—6020 5540	0.94—1.07 0.99	5200—9200 6950
	18	♀	79—91 84	4360—5100 4730	0.93—1.06 0.99	4600—12000 7550
Appelberg	11	♂	94—112 104	4950—5720 5420	0.90—1.08 1	3900—6700 5030
	11	♀	81—96 87	4050—4820 4570	0.91—1.13 1	4400—7000 5950
Forssell	10	♂	85—108 101	4592—5240 4992	0.92—1.06 1.02	4800—7100 5610
	10	♀	85—98 89	4032—5388 4587	0.89—1.05 0.98	5300—7400 6270
Tötterman	11	♂	97—108 102.6	4608—5232 4972	0.98—1.06 1.03	4700—7400 5709
	11	♀	85—98 90.3	4032—5120 4545	0.91—1.05 0.99	5500—7400 6536
Virkkunen	10	♂	86—109 99.4	4464—5960 4927	0.84—1.1 0.99	3800—7900 6559
	10	♀	81—90 86.1	3968—4560 4315	0.93—1.06 0.99	3800—8900 5390

<sup>1</sup> 13 Fälle.<sup>2</sup> 10 Fälle.<sup>3</sup> Die Werte des weissen Blutbildes umfassen 10 ♂ und 10 ♀.

## 5.

werte des normalen Blutbildes.

Neutr. Stab.	Neutr. Segm.	Basoph.	Eosinoph.	Mono.	Lymphoz.	Ret. %
3—5 4	51—67 63	0—1 0.5	2—4 3	4—8 6	21—35 23	
48.8—71.3 60		0—1.3 0.6	0.3—3 1.6	4—10.3 7.1	22.8—38.5 30.6	
48.5—68.3 58.4		0—1.3 0.6	0.2—3.1 1.6	3.2—9.1 6.1	24.7—42.5 33.6	
41.8—61.4 56.6		1—2.4 1.7	3.2—3.2 3.2	8.4—10 9.2	26.6—42.6 34.6	
49.2—67.2 57.7		0—1.4 0.72	1.2—5.2 2.87	4.4—11.6 9.36	23.2—38 29.7	
38—67.1 55		0—1.8 0.9	0.7—5.5 2.8	4.4—9.2 6.6	25—41.7 34.7	
53.4—67.5 59.2		0.1—0.6 0.4	0.6—2.8 1.5	3.8—8 5.4	25.2—40 33.5	
0.5—3 1.25	49.25—77 61.8	0—2 0.7	1.25—4.5 2.9	3.75—7.75 5.75	16.75—41.25 27.6	0—0.5 0.24
0—4 2.9	56.5—74 64.9	0—1 0.45	0.5—4.25 2.8	3—8.25 5.1	16.25—36 24.8	0.1—0.6 0.22
0—5.5 2.5	36.5—72 53.7	0—1.5 0.54	0.5—6.5 3.32	4.5—10 7.5	18—40 32.4	0.1—0.5 0.24
0—5 2.86	40—74 59	0—1.5 0.77	0—4.5 1.95	2.5—14.5 7.2	16—43 28.4	0.1—0.6 0.24
1.2—3.6 2.3	50—68.6 60.8	0—1.6 0.6	0.2—3.6 2.1	1.4—7.4 4.5	21.0—38.2 29.7	0.2—0.8 0.4
0.4—4.6 3	45—63.6 54.8	0—1 0.6	0.4—3 1.9	1—10.8 5.3	22—43.4 34.6	0.2—1 0.4



geteilten kasuistischen Beobachtungen, bei denen er diesen Krankheitszustand auf eine besondere spezifische Infektion zurückführen wollte. Heute nehmen wir an, dass es sich meist um eine »anaphylaktische Krise des myeloischen Gewebes« handelt (v. BAYER, BOCK, ROHR), deren klinische Symptome verhältnismässig genau umgrenzt sind und die aus mehreren verschiedenen Ursachen ausbrechen kann. Nachdem vor 10 Jahren fast gleichzeitig auf mehreren Seiten (COSTEN, KRACKE und PARKER, MADISON und SQUIER, VIDEBECH) festgestellt worden war, dass die Pyrazolonderivate und mit ihnen verwandte, früher als harmlos betrachtete Medikamente einen Ausbruch der Krise bewirken können, sind im Schrifttum die die Granulozytopenie hervorrufenden medikamentöstenoxischen Faktoren Gegenstand besonderer Aufmerksamkeit gewesen. Ausser ihnen wird den Infektionen in der Pathogenese der Granulozytopenie eine wichtige Rolle zugeschrieben. Ferner scheinen auch mehrere andere, seltenere Faktoren die Entstehung der Krise herbeiführen zu können, wie endokrine Störungen (JACKSON und Mitarbeiter, THOMSON, WILKINSON und ISRAELS) und alimentäre Faktoren (CASTLEDEN, FETTES und WHITBY). Es ist möglich, dass die Granulozytopenie, wie EDSTRÖM bemerkt, meist durch die kombinierte Wirkung mehrerer verschiedener Ursachen entsteht. Von der Kompliziertheit der Pathogenese zeugen Fälle, bei denen der Patient auf die gleiche (Angina-) Infektion bald mit Granulozytopenie, bald mit einer normalen Leukozytose reagiert (EHRMANN und PREUSS).

Für das klinische Krankheitsbild der Krise ist eine Neigung zu Infektionen charakteristisch, die leicht einen nekrotisierenden und malignen Charakter annehmen. Hin und wieder ist der Entzündungsprozess in die Tonsillen lokalisiert. Schon in seinem ersten Bericht über die Granulozytopenie erwähnt SCHULTZ die dazu gehörende nekrotisierende Tonsillitis. Und nach der Ansicht FRIEDEMANN's (1923) ist die Angina so kennzeichnend für diesen Krankheitszustand, dass er für sie den Namen »Angina agranulocytotica« vorschlug. Die spätere Erfahrung hat gezeigt, dass die Tonsillitis kein obligatorisches Symptom im Zusammenhang mit der Granulozytopenie-Krise ist, obgleich sie sehr gewöhnlich auftritt. So kam sie in der 330 Fälle umfassenden Statistik von TAUSSIG und SCHNOEBELEN zu 74 % vor.

Es hat sich als schwierig erwiesen, aufzuklären, welchen Anteil



daneben verdächtigten Medikamentes gerichtet. Die Beurteilung der Behandlungsergebnisse ist jedoch schwierig, weil auch spontane Heilungen vorkommen. Sogar schwere Fälle, in denen ausge dehnte Nekrosen vorliegen, können spontan heilen. Es ist eine allgemeine Erfahrung, dass die Ergebnisse bei der Anwendung verschiedenartiger die Myelopoese anregender Behandlungsverfahren unsicher sind. Nach wie vor ist die Prognose der Granulozytopenie-Krise bei der Anwendung der vorerwähnten Therapie als schlecht zu betrachten. Das zeigen deutlich die 1939 von JACKSON und TIGHE aus dem Schrifttum zusammengestellten 390 Fälle, in denen das Mortalitätsprozent 45.6 betrug.

Eine andere Therapie richtet sich auf die Bekämpfung des Infektionszustands, der sich im Zusammenhang mit der Krise entwickelt. Dabei kommen vor allem die Granulozytopenie-Anginen in Betracht, also Patienten, bei denen die sich in Verbindung mit der Krise entwickelnde Infektion in die Gaumenmandeln lokalisiert ist. Auch in solchen Fällen hat man die Hauptaufmerksamkeit der Stimulation der Hämatopoese zugewandt. In der Literatur finden sich nur wenig Angaben über Patienten, bei denen die Behandlungsmassnahmen ausschliesslich oder vorzugsweise auf die Heilung der Tonsillitis gerichtet worden sind. Zur Bekämpfung oder Überwindung der Tonsillitisinfektion können bekanntlich verschiedene Methoden angewandt werden. IVES hat einen und MENDES DE LEON zwei Fälle veröffentlicht, in denen eine schwere Granulozytopenie-Angina nach massiver Sulfanilamidmedikation zur Heilung gelangte. Ausser medikamentöser Behandlung kann auch chirurgische Therapie, d. h. die Ausführung einer Tonsillektomie während der Krise, in Frage kommen. Auch diesem Verfahren ist im Schrifttum nur wenig Beachtung geschenkt worden. In einigen Arbeiten wird die Möglichkeit der Tonsillektomie bei der Granulozytopenie-Angina behandelt, aber vor chirurgischen Eingriffen während der Krise gewarnt, eine Auffassung, die damit begründet wird, dass die Heilung der Operationswunden in Ermangelung von Granulozyten schlecht sei. Es sei eine schwere Nekrotisierung der Schnittstellen zu erwarten, wodurch die Entstehung eines septischen Zustands befördert werde. Diese Ansicht äussert z. B. SCHULTZ noch in einem 1939 erschienenen Aufsatz. Auf demselben Standpunkt stehen auch KRÜGER, SEILER und STRASSER. Diese Auffassung stützt sich jedoch auf keinerlei systematische Unter-

suchungen. Vielleicht gründet sie sich auf Fälle, in denen die Krise nach verschiedenartigen chirurgischen Eingriffen zum Vorschein gekommen ist und auf die u. a. KRÜGER hinweist. So hat HILL über zwei Fälle berichtet, die unmittelbar nach einer Zahnextraktion ausbrachen. Vor der Massnahme war das Blutbild in dem einen dieser Fälle normal, in dem anderen wird nichts darüber erwähnt. BROGSITTER und v. KRESS haben einen Patienten beschrieben, bei dem sich nach der Tonsillektomie wegen einer rezidivierenden Tonsillitis eine letale Granulozytopenie entwickelte. Auch SEIFERT hat über einen ähnlichen Fall berichtet. Von den Blutbildern vor der Tonsillektomie bei diesen Patienten ist nichts gesagt.

Für die heute herrschende Anschauung ist eine im Arch. Int. Medizin 1940 erschienene Besprechung kennzeichnend, die REIMANN über eine von HOLSTI, MEURMAN und mir veröffentlichte vorläufige Mitteilung über einen Fall (Nr. 21) gab. Er schreibt: »As examples of the type of misleading and uncontrolled information, which editors of medical journals occasionally accept are papers in which the authors recommend tonsillectomy for the treatment of granulocytopenic angina.«

In der früheren Literatur habe ich einige mit Tonsillektomie behandelte Fälle von Granulozytopenie-Angina angetroffen. Ich habe diese Fälle in Tabelle 6 zusammengestellt und versucht, ihren Schweregrad auf Grund der klinischen Symptome und der Blutveränderungen zu analysieren. Bei dem von MARSCHIK beschriebenen Patienten wurde eine einseitige Tonsillektomie und eine Jugularisresektion ausgeführt, wobei das Blutbild 900 Leukozyten und eine starke Neutropenie zeigte. Die Heilung ging schnell vor sich, aber einige Wochen später bekam der Patient ein Rezidiv auf der anderen Seite. Auch diesmal wurde eine Tonsillektomie gemacht, doch war die Wirkung nicht so deutlich. Der Patient wurde zwar geheilt, aber erst nach verhältnismässig langer Zeit, nachdem er auch Röntgentherapie in das Mark der langen Knochen bekommen hatte. Bei der Patientin von WESTERGREN zeigte das Blutbild eine deutliche Remission, aber es entwickelte sich innerhalb einer Woche nach der Operation eine letale Pneumonie. KINDLER hat die Tonsillektomie bei einem und GRAHE bei zwei Patienten ausgeführt, bei denen ausser der Granulozytopenie eine schwere Anämie auftrat. In

Tabelle 6.

Mit Tonsillektomie behandelte Fälle von Angina granulocytopenica.

Autor	Jahr	Art der Krise	Komplikationen u. Nebenkrankheiten	Zeitpunkt der Tonsillektomie	Erfolg
MARSHIK	1931	Schwer	Thrombosis v. jugularis	Nicht erwähnt	Geheilt
WESTERGREN	1933	»	Pneumonia. Polyarthrit	17 Tage nach der Krise	Gestorben
GRAHE	1938	»	Pneumonia. Mastoiditis	3 » » » »	Gestorben
		»	Pneumonia. Schwere Dentalinfektion	14 » » » »	»
		»	Pneumonia. Anaemia gravis	21 » » » »	»
		»	Grosse Nekrosen d. Wangenschleimhaut	28 » » » »	»
		»	Mastoiditis (Anthrotomia)	11 » » » »	Geheilt
		Leicht	—	21 » » » »	»
		Schwer	Otitis media. Anaemia gravis	Nicht erwähnt	»
KINDLER	1938	Leicht	Grosse Nekrosen d. Wangenschleimhaut u. d. Zahnfleisches	» »	»
		»	Schwere Anämie	» »	(2 Mon. später an Leukämie gestorben.)
		Schwer	Nach Tonsillektomie und Zahnextraktion Nekrosen und Sequestrierung des harten Gaumens	» »	Geheilt
LINCK	1938	Leicht	Nekrosen in den Operationswunden	8 Tage nach der Krise	»
		Schwer	» »	Nicht erwähnt	»
VIRKKUNEN	1943	»	Bronchitis	6 Tage nach der Krise	»
		Leicht	—	10 » » » »	»
		»	—	12 » » » »	»
		Schwer	Pneum. (Heilungsstadium). Enteritis	3 » » » »	»
		»	Pneumonia l. a. Nephrosis	8 » » » »	Gestorben
		»	Pneumonia Mediastinitis	6 » » » »	»

diesen Fällen handelte es sich nicht um echte Granulozytopenie. Über den ersteren Fall haben auch VOIT und LANDES in einer Arbeit berichtet. Es zeigte sich bei dem Patienten vor der Tonsillektomie das Krankheitsbild der Panmyelophthise, und er starb ein paar Monate später, wo das Blutbild und die Obduktion die für die akute Myeloblastenleukämie charakteristischen Zeichen hervortreten liessen. Bei den Patienten von GRAHE fehlen genaue Angaben über den hämatopoetischen Zustand. Sein Material enthält ausserdem einige schwere septische Fälle, bei denen zu der Tonsillitis hinzu auch andere entzündliche Lokalisationen auftraten. In den beiden Fällen von LINCK nahmen die Blutveränderungen nach der Operation zuerst zu, und in den Wundflächen entwickelten sich Nekrosen. Die Patienten genasen allmählich im Lauf von ein paar Wochen, nachdem sie Bluttransfusionen bekommen hatten.

LINCK hat auch über einen Fall berichtet, in dem der eigentliche Krisenzustand unter konservativer Behandlung sich verbesserte, aber ein fortbestehender leichter granulozytopenischer Zustand zurückblieb, der 3 Monate später nach Tonsillektomie völlig verschwand. Der Patient hatte keine ausgesprochenen Symptome von Tonsillitis, aber bei der histologischen Untersuchung wurden in den Mandeln deutliche entzündliche Veränderungen festgestellt. Sehr ähnlich ist der Fall von v. GARDOS. Die betreffende 3jährige Patientin hatte die eigentliche Krise überstanden, doch war ein chronischer granulozytopenischer Zustand zurückgeblieben, mit dem sich eine andauernde Subfebrilität verband. Nachdem der Zustand 4 Monate unverändert angehalten hatte, wurde eine Tonsillektomie ausgeführt. Danach wurde die Patientin klinisch und hämatologisch geheilt. Derartige Beobachtungen sind auch im Hinblick auf die Diagnose von Fokalherden wichtig, da sie zeigen, dass solche Herde auch Leuko- und Granulozytopenie hervorrufen können.

In einigen Veröffentlichungen ist die Tonsillektomie mit gewissem Vorbehalt empfohlen worden. ZANGE äussert die Ansicht, dass es bei den Granulozytopenie-Anginen wichtig sei, zu analysieren, ob die Tonsillitis primär oder sekundär ist. Im ersteren Fall befürwortet er die Tonsillektomie. Ist die Tonsillitis dagegen sekundär infolge von Blutveränderungen entwickelt, so betrachtet er eine Operation wegen der Ausbreitung der Nekrosen und wegen der

Sepsisgefahr als kontraindiziert. In solchen Fällen sollte seiner Meinung nach zuerst durch eine die Hämatopoese stimulierende Therapie die Krise überwunden und erst danach die Tonsillektomie vorgenommen werden, wo die Sepsisgefahr bei möglichen neuen Krisen geringer sei. Er hat einmal nach einer Krise die Tonsillektomie ausgeführt, die der Patient gut vertrug. Der Auffassung ZANGE's sind u. a. KIEBEL und WESSELY beigetreten. Auch MATH empfiehlt die Tonsillektomie als prophylaktische Massnahme in rezidivierenden Fällen während der Intervalle zwischen den Krisen.

Aus dem obigen Überblick geht hervor, dass die im Schrifttum vorgelegten Auffassungen über die Möglichkeiten der Tonsillektomie bei Fällen mit Granulozytopenie-Angina einander widersprechen. Über diesen Eingriff liegen nur wenige und teilweise mangelhafte klinische Erfahrungen vor.

## 2. Eigene Untersuchungen.

### a) Kasuistik.

Mein Material an Granulozytopenic-Anginen umfasst infolge der Seltenheit der Krankheit nur 6 Fälle. Die Patienten stammen aus der Oto-Laryng. Klinik, dem Epidemiekrankenhaus der Stadt Helsinki und aus dem Privatkrankenhaus Mehiläinen. Im folgenden gebe ich die Krankenberichte der Patienten.

Fall 21.<sup>1</sup> (Abb. 7.) 36jährige Frau. In der Oto-Laryng. Klinik 31. 5.—8. 6. 1938. (Journ.-Nr. 495/38.) Auf der klinischen Abteilung der med. Poliklinik 9. 6.—11. 6. 1938. (Journ.-Nr. 111/38.)

Anamnese: In der Familie der Patientin kommen mehrere Fälle von perniziöser wie hypochromer Anämie vor. Bei einer Tochter trat Granulozytopenie auf. Die Patientin selbst war als Kind im allgemeinen gesund, aber schwächlich und blass. Etwa 12 Jahre wiederholt Anginen, anfangs mehrmals im Jahre. Zwischendurch erschienen sie seltener, aber in den zwei letzten Jahren wieder häufiger. Die Angina dauerte jedesmal ungefähr eine Woche. Peritonsillarabszesse sind nicht vorhanden gewesen. Im Januar 1938 ab und zu leichte Rachenschmerzen, wobei keine Temperatursteigerung. In den zwei letzten Monaten besonders starke Müdigkeit. Oft Kopfschmerz, weshalb Amidopyrin zusammen etwa 7—8 g im

<sup>1</sup> Diesen Fall haben Ö. HOLSTI, Y. MEURMAN und ich als vorläufige Mitteilung veröffentlicht. Siehe Acta Med. Scand. Vol. CIII, 1940, S. 431.

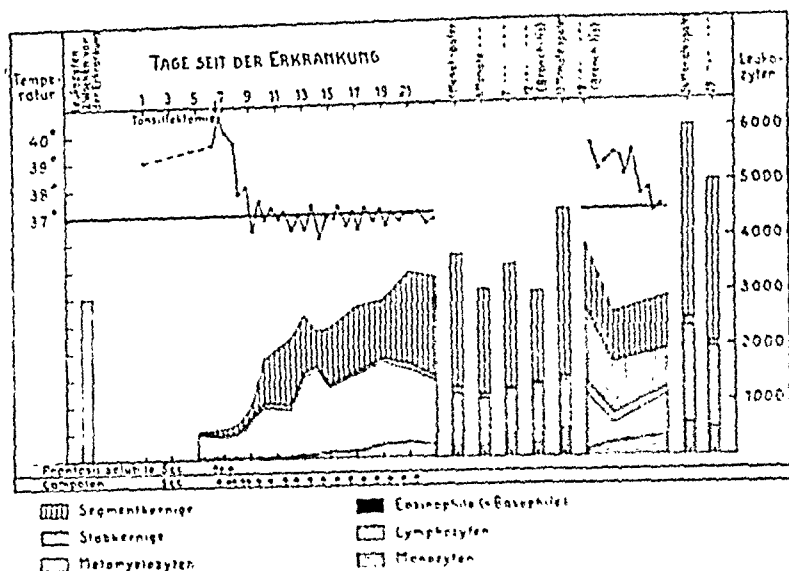


Abb. 7. Fall 21.

Laufe von 4 Monaten genommen wurde. Am 26. 5. 1938 erkrankte die Patientin mit Rachenschmerzen und Fieber. (Die Tochter hatte ein paar Tage vorher ebenfalls eine Angina bekommen.) Am Tage nach der Erkrankung Schüttelfrost, der später bis zur Aufnahme in die Klinik 2—3 mal täglich auftrat. Fieber zwischen  $38^{\circ}$  und  $39^{\circ}$ . Vom Anfang der Angina an hat die Patientin unregelmässig Sulfanilamid, im ganzen 5—6 g, genommen.

St. praesens am 31. 5. 1938: Körperbau und Ernährungszustand gewöhnlich. Allgemeinbefinden schlecht. Temp.  $39,6^{\circ}$ . Die Patientin ist blass und hat Kaltschweiss. Gl. thyroidea gewöhnlich. Sensorium klar. Vom Nervensystem nichts zu erwähnen. Puls frequent, weich. Herz o. B. In beiden Lungen trockenes und zahes Rasseln. Mundschleimhaut trocken, gerötet, keine Nekrosen. Beide Tonsillen stark gerötet, von der Grösse einer grossen Daumenkuppe. Auf der Oberfläche der Tonsillen weder Beläge noch Nekrosen. Unterhalb der beiden Mandibularwinkel in der Jugularisregion einige fast fingerendgrosse, leicht schmerzhaft Lymphknoten. Bauch o. B. Harn: Eiw. —. Fäzes: Wurmeier —, Wagner —, WR —. Kalm —. Blutkultur —. Sternalpunktat in Tabelle 14. Blutbilder in Tabelle 15.

Decursus: Am 31. 5. 1938 Tonsillectomia bilateralis. Die Tonsillen werden mit der Kapsel unter Schonung der Gaumenbögen abgelöst. Sie lösen sich leicht los. Narbenadhärenzen oder peritonsilläre Eiterherde sind nicht festzustellen. Wundflächen glatt. Die Tonsillen daumenspitzen-gross. Darin keine makroskopischen Veränderungen. (Bei der von Prof. Ö. HOLSTI ausgeführten histologischen Untersuchung waren kleine nekrotische Herden und Zeichen eines frischen ulzerativen sowie eines vernar-



benden Prozesses zu konstatieren.) Abgesehen von der Tonsillektomie, wurde die Patientin mit Prontosil (an zwei Tagen) und Campolon behandelt (siehe Abb. 7). Nach der Operation verschwand das Fieber in ein paar Tagen, und der Allgemeinzustand verbesserte sich schnell. Die Heilung der Wundflächen schritt normal fort.

Später nach der Operation war das Befinden der Patientin gut. Das Allgemeinbefinden war besser als in ein paar Jahren vor der Tonsillektomie. Im Frühjahr 1939 erkrankte die Patientin an einer fieberhaften Bronchitisinfektion, von der sie sich schnell erholte. Im November 1939 bekam sie wieder eine Bronchitis mit hohem Fieber. Zwei andere Familienmitglieder hatten etwas vorher »Influenza« gehabt. Diesmal dauerte die Infektion eine Woche. Bei beiden Infektionen traten die Granulozytopenie-Veränderungen im Blute in leichter Form erneut auf. Laut Mitteilung im Dezember 1942 ist die Patientin andauernd vollständig gesund.

**Epikrise:** Eine 36jährige Frau, in deren Familie zahlreich Blutkrankheiten vorgekommen sind und die selbst eine dauernde leichte Leuko- und Neutropenie hat. In der früheren Anamnese eine rezidivierende Tonsillitis. Sie erkrankte an einer Granulozytopenic-Angina und hatte vor der Erkrankung im Laufe von 4 Monaten 7—8 g Amidopyrin und während der Krise 5—6 g Sulfanilamid genommen. Eine Tonsillektomie wurde 7 Tage nach dem Beginn der Krise ausgeführt. Die Patienten genas schnell. Sie hatte auch Leberinjektionen und geringe Mengen Prontosil bekommen. Später, im Anschluss an zwei Respirationsinfektionen, kam es wieder zu leichten granulozytopenischen Veränderungen im Blute.

**Fall 22.** (Abb. 8.) 18jähriger Arbeiter. In der Oto-Laryng. Klinik 15. 12.—20. 12. 1938. (Journ.-Nr. 1135.)

**Anamnese:** Über die Familie ist nichts zu bemerken. Der Patient ist im allgemeinen gesund gewesen. Weder Neigung zu Angina noch zu anderen Infektionen. Am 6. 12. 1938 erkrankte er mit Rachenschmerzen und Fieber ad 38.5°. Während der folgenden Tagen verblieb der Zustand unverändert, weshalb der Pat. sich am 13. 12. in die Poliklinik des städtischen Mariakrankenhauses begab. Dort wurde wegen Verdachts auf einen Peritonsillarabszess eine Inzision gemacht. Es kam jedoch kein Eiter. Von dort wurde Pat. in das Epidemiekrankenhaus gesandt, wo er zwei Tage war und insgesamt 3 g Sulfanilamid bekam. Am 15. 12. wurde er weiter zur Tonsillektomie in die Universitäts-Ohrenklinik geschickt.

**St. praesens** am 15. 12. 1938: Kräftig gebauter Mann, dessen Allgemeinzustand nicht besonders belastet ist. Temp. 38.4°. Unterhalb der Kieferwinkel beiderseits eine daumenspitzen-grosse, leicht empfindliche Drüse, anderswo keine Drüsen-schwellungen. Innere Organe o. B. Milzdämpfung gewöhnlich. Harn: Eiw.—. Nyl.—. WR—. Kahn—. Ster-nalpunktate in Tabelle 14. Blutbilder in Tabelle 15.

Öffnen des Mundes etwas erschwert. Mundschleimhaut gerötet, ziemlich trocken. Linke Rachenmandel reichlich taubeneigross, gedunsen, gerötet, schleimbedeckt, stark medialwärts verschoben, so dass sich ihre Innenfläche bis zur Mittellinie erstreckt. Das peritonsilläre Gewebe lateralwärts und oberhalb der Tonsille bedeutend angeschwollen und gerötet. Oberhalb-lateral von der Tonsille eine  $\frac{3}{4}$  cm lange Inzisionswunde, aus der ziemlich reichlich gelber, stinkender Eiter kommt. Als die Wunde aspiriert wird, scheint es, als sei der Schnitt gerade in das Tonsillengewebe gegangen. Uvula rot, ödematös, etwas nach rechts gewandt. Auch der

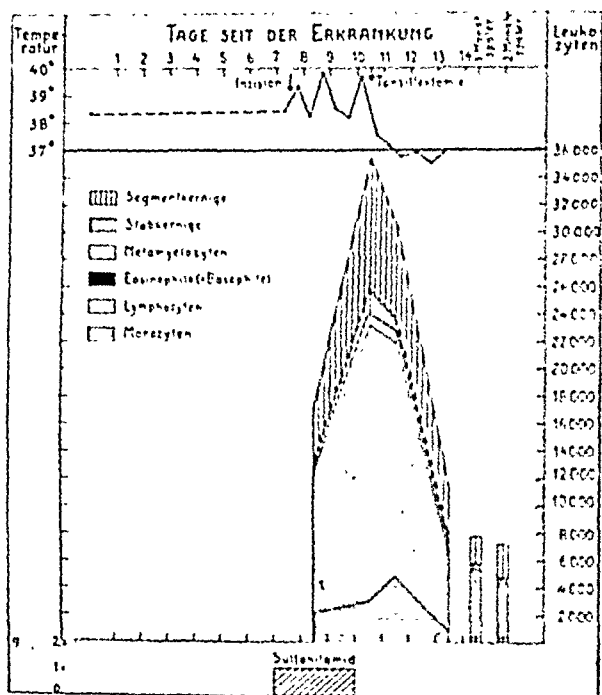


Abb. 8. Fall 22.

vordere Gaumenbogen stark geschwollen und gerötet. Rechte Tonsille fingerspitzengross, etwas gerötet, darin ein paar Flecke. Keine Peritonsillitis. Die Hinterwand des Rachens etwas geschwollen, gerötet, schleimig.

Decursus: 16. 12. 1938 Tonsillektomie. Die linke Tonsille wird mit der Kapsel unter Schonung der Gaumenbögen abgelöst. Die Tonsille einigermaßen mit ihrer Umgebung verwachsen. Das peritonsilläre Gewebe fester als gewöhnlich, geschwollen. Ein Peritonsillarabszess ist nicht zu finden. Tonsille pflaumengross, Gewebe spröde, rötlich. Im Innern der Tonsille keine Narben. Der in den vorderen Gaumenbogen gelegte Schnitt geht geradeaus in das Tonsillengewebe durch die Tonsille bis in den hin-

teren Gaumenbogen. Das Tonsillengewebe in der Wand des Schnittkanals belegt, etwas nekrotisch. Die rechte Tonsille fingerspitzenlang, löst sich leicht mit der Kapsel ab. Gewebe von normalem Aussehen.

Nach der Operation verschwand das Fieber schnell, und der Patient befand sich wohl. Die Wundflächen heilten normal.

Nachuntersuchungen am 18. 1. 1939 und 15. 2. 1939. Das Befinden des Patienten war während der ganzen Zeit gut. Operationswunden gut geheilt.

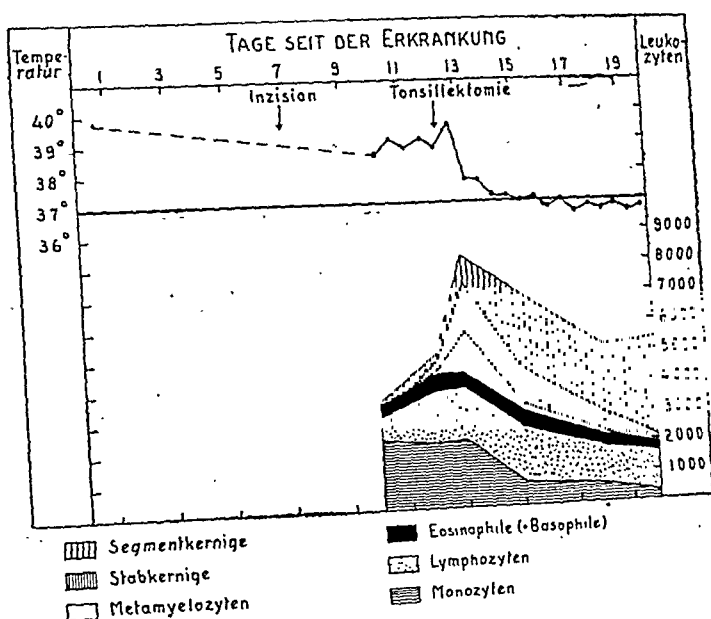


Abb. 9. Fall 23.

**Epikrise:** Ein 18jähriger Mann, der zum erstenmal an Angina erkrankte. Eine Woche später wurde wegen Verdachts auf einen Peritonsillarabszess eine Inzision gemacht, aber es wurde kein Eiter angetroffen. 10 Tage nach der Erkrankung wurde eine Tonsillektomie ausgeführt, wonach die im Bluthild erschienene neutropenische Leukozytose schnell verschwand.

**Fall 23.** (Abb. 9.) 17jährige Hausangestellte. In der Oto-Laryng. Klinik 20. 12.—29. 12. 1938. (Journ.-Nr. 1145.)

**Anamnese:** Familiär o. B. Die Patientin war als Kind gesund. In 3 Jahren erkrankte sie mehrmals an fieberhaften Anginen, die je einige Tage dauerten. Peritonsillarabszesse sind nicht aufgetreten. Vom 18. 10.—1. 12. 1938 wurde sie in der Dermatologischen Universitätsklinik behandelt. Die Diagnose lautete: Lues recens (Infectio extragenitalis). Erosio indurata labii inferioris. Roseola trunci. Adenopathia submandibularis. 3 Wochen vor der Aufnahme in die Klinik hatte die Patientin einen klein-

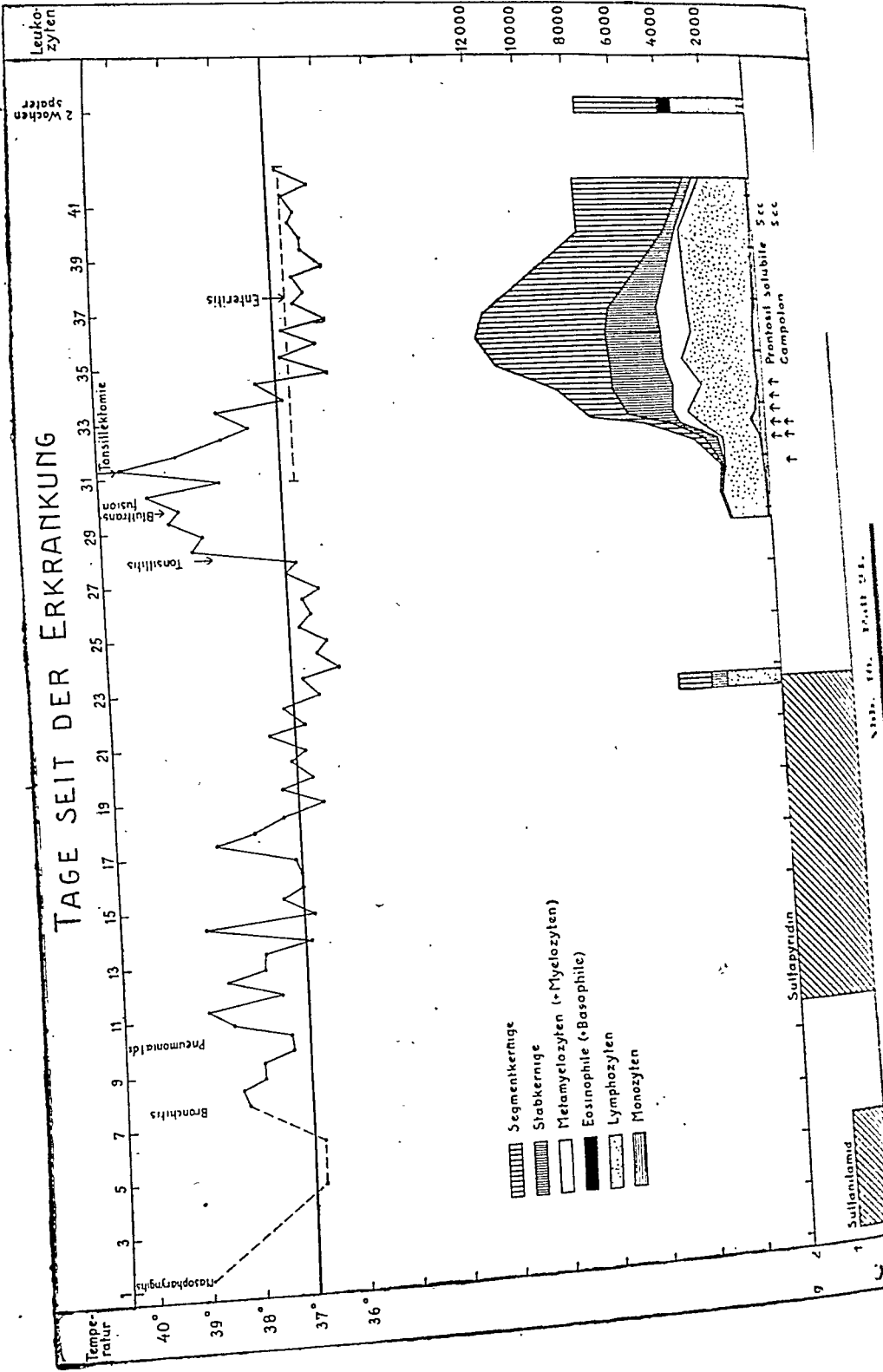
fingerspitzengrossen Knoten an der Unterlippe bekommen. WR + und Kahn +. Kombinierte Neosalvarsan-Wismutkur (4.5 g Neosalvarsan und 2 g Wismut). Am 9. 12. 1938 (etwa eine Woche nach Abschluss der Kur) stellten sich Fieber und Rachenschmerzen ein. Der Zustand verblieb unverändert, weshalb die Patientin am 15. 12. die Poliklinik des städtischen Mariakrankenhauses besuchte. Hier wurde wegen Verdachts auf einen Peritonsillarabszess eine Inzision gemacht, aber Eiterherde wurden nicht angetroffen. Der Patientin wurde ein Medikament (Sulfanilamid?) verordnet, das sie zu Hause nehmen sollte. Wieviel sie davon gebraucht hat, ist nicht festzustellen. Am 18. 12. kam sie in das Epidemiekrankenhaus, von wo sie weiter zur Operation in die Ohrenklinik geschickt wurde.

St. p r a e s e n s am 20. 12. 1938: Konstitution gewöhnlich. Allgemeinzustand mässig. Temp. 38.6°. Keine Drüsenschwellungen. Nervensystem, Herz, Lungen und Bauch o. B. Öffnen des Mundes etwas erschwert. Foetor ex ore. Mundschleimhaut normal. Linke Tonsille gerötet, angeschwollen, fast pflaumengross. In der Höhe ihres oberen Poles ein querlaufender, 1.5 cm langer Schnitt, durch den graues, bedecktes nekrotisches Gewebe zu sehen ist. Aus dem Schnitt kein nennenswertes Sekret. In der Umgebung des Schnittes oberhalb und lateral von der Tonsille eine peritonsilläre Anschwellung, in deren Bereich die Schleimhaut gerötet ist. Die angeschwollene Tonsille verschiebt die gerötete und gedunsene Uvula nach rechts. Die rechte Tonsille fingerspitzengross, leicht gerötet. Auf ihrer Oberfläche Schleim. Sternalpunktate in Tabelle 14. Blutbilder in Tabelle 15.

D e c u r s u s: 21. 12. 1938 Tonsillektomie. Es wird festgestellt, dass der Inzisionsschnitt das linke Tonsillengewebe getroffen und die Tonsille so durchtrennt hat, dass oberhalb der Schnittstelle ein bohnergrosses Stück verblieben ist. Der unterhalb befindliche reichlich daumenspitzen-grosse Teil der Tonsille wird mit der Kapsel entfernt. Ein Peritonsillarabszess ist nicht zu entdecken. Der oberhalb der Schnittstelle gelegene Teil der Tonsille wird weggenommen. Die Gaumenbögen bleiben intakt. Die rechte Tonsille ist fingerspitzengross und löst sich leicht ab. Gewebe der linken Tonsille spröde. Auf seiner medialen Oberfläche kleine nekrotische Gebiete.

Nach der Operation hat sich das Befinden der Patientin sowohl subjektiv als objektiv schnell gebessert. Zustand bei der Entlassung aus der Klinik am 29. 12. 1938 gut. Wundflächen sehr sauber. Keine nennenswerten Schlingbeschwerden.

E p i k r i s e: Eine 17jährige Frau, die früher eine rezidivierende Tonsillitis gehabt hatte, bekam wegen einer Luesinfektion eine kombinierte Neosalvarsan-Wismutkur. Eine Woche später erkrankte sie an einer Angina. 7 Tage danach wurde wegen Verdachts auf einen Peritonsillarabszess eine Inzision gemacht, aber es wurde kein Eiter angetroffen. 6 Tage später wurde eine Granulocytopenie festgestellt und eine Tonsillektomie ausgeführt, worauf die Blutveränderungen schnell verschwanden.



Fall 24.<sup>1</sup> (Abb. 10.) 13jähriges Schulmädchen. Im Privatkrankenhaus Mehiläinen 11. 8.—31. 8. 1939 (Nr. 2171.)

**Anamnese:** Der Vater und seine Schwester leiden an chronischer Polyarthrit. Vor 3 Jahren hatte die Patientin eine mehrere Monate dauernde Fussgelenkentzündung, die als tuberkulös verdächtigt wurde. Röntgenologisch wies das Gelenk keine Veränderungen auf. Pirquet war negativ. Im Dezember 1938 bekam sie Masern, die ohne Komplikationen heilten. Im Frühjahr 1939 oft leichter Husten. Anginen sind früher nie vorgekommen.

Am 20. 7. 1939 erkrankte die Patientin an einer akuten Naso-pharyngitis-Infektion, wobei das Fieber auf 39° stieg. Das Fieber sank in ein paar Tagen, aber einige Tage später rezidierte es. Dabei wurde Bronchitis festgestellt. Ausser Husten hatte die Patientin wiederholt leichtes Nasenbluten. Ein paar Tage danach waren in der Spitze der rechten Lunge auf eine Pneumonie hinweisende Zeichen zu konstatieren. Das Fieber sank allmählich, aber in der Spitze der rechten Lunge bestand fortgesetzt eine Dämpfung und feuchtes verhärtetes Rasseln. Bei zwei Untersuchungen fand man in dem Auswurf keine Tuberkelbazillen. Seit dem Beginn der Erkrankung hat die Patientin 5 g Sulfanilamid und 22 g Sulfapyridin genommen.

**St. praesens** am 11. 8. 1939: Körperbau gewöhnlich. Das subkutane Gewebe ziemlich spärlich, nicht reduziert. Keine Drüsenschwellungen. Nervensystem: o. B. Herz: o. B. Lungen: Im Spitzenteil der rechten Lunge eine deutliche relative Dämpfung und reichlich feuchtes, verhärtetes Rasseln. Rachen und Bauch: o. B. Harn: Eiw. —. Nyl. —. Fäzes: Wurmeier —. Wagner —. Sputa: Tbc —. Sternalpunktate in Tabelle 14. Blutbilder in Tabelle 15.

**Röntgenuntersuchung der Lungen:** Die rechte Spitze klärt sich beim Husten nicht ganz auf. Die Diaphragmen bewegen sich gut. Die Sinus frei. Unterhalb des rechten Schlüsselbeines ein etwa gänseeigrosser Ringschatten (eine Höhle?) näher bei der Hinterwand. In der rechten Spitze Fasern und Flecke. Das Mediastinum mehr rechts als gewöhnlich. Das Herz o. B.

**Decursus:** Am 11.—14. 8. nahmen die Lungenveränderungen allmählich ab, so dass nur bronchovesikuläres Atmen und spärlich zähes Rasseln zu hören war.

Am 16. 8. wurde eine Magenspülung ausgeführt, um in der Spülflüssigkeit nach Tuberkelbazillen zu suchen. Am Abend desselben Tages stieg das Fieber plötzlich auf 38,8°, und in der folgenden Nacht fühlte die Patientin Schmerzen im Rachen.

17. 8. Rachen diffus gerötet, ebenso die Mundschleimhaut. Beläge sind nicht festzustellen, aber auf der linken Rachenmandel findet sich eine ganz dünne netzförmige Haut. Unterlippe geschwollen. **Röntgenuntersuchung:** Die rechte Spitze klärt sich beim Husten nicht auf.

<sup>1</sup> Diesen Fall hat mir Dr. med. P. NOPONEN freundlichst überlassen, wofür ich ihm meinen besten Dank ausspreche.

Beweglichkeit des rechten Diaphragmas herabgesetzt. Ringschatten unterhalb der rechten Clavicula verkleinert.

18. 8. An der rechten Wange ein und auf der linken Tonsille einige stecknadelkopfgrosse Flecke. Rötung der rechten Tonsille vermindert. Zunge gerötet. Allgemeinzustand der Patientin gut. Es wurde eine Bluttransfusion gemacht.

19. 8. Befinden am Morgen gut. Auf beiden Rachenmandeln einige Flecke. Links unterhalb des Kieferwinkels eine kleinfingerspitzen-grosse, etwas empfindliche Drüse. Später am Tage Schüttelfrost. Allgemeinzustand schlechter, Patientin sehr müde. Rötung und Schwellung der Tonsillen gesteigert. Die Patientin bekam eine Diarrhöe, die, allmählich nachlassend, 10 Tage lang bestand. Darauf wurde nach Konsultation mit Prof. Ö. HOLSTI und Chefarzt Dozent V. RANTASALO am 19. 8. abends eine Tonsillektomie ausgeführt (Dr. med. P. KOSKINEN). Unmittelbar nach der Operation sank das Fieber, und der Allgemeinzustand besserte sich schnell. Die Beläge auf den Operationswunden waren nicht grösser als gewöhnlich, und sie verschwanden in einigen Tagen.

12. 9. 1939. Nachuntersuchung. Befinden subjektiv und objektiv gut.

**Epikrise:** Ein 13jähriges Mädchen, das an einer langwierigen Pneumonie und, als sich diese im Heilungsstadium befand, an einer Granulozytopenie-Angina erkrankte. 4 Tage später wurde eine Tonsillektomie ausgeführt, worauf die Blutveränderungen schnell verschwanden. Die Patientin hatte auch kleine Mengen Prontosil und Campolon bekommen.

Fall 25.<sup>1</sup> (Abb. 11.) 49jähriger Dekorationsmaler. Im Epidemiekrankenhaus 22. 2.—27. 2. 1941. (Journ.-Nr. 564/41.)

**Anamnese:** (Infolge des mitgenommenen Zustands des Patienten blieb die Anamnese bei der Ankunft im Krankenhaus unvollständig, und die früheren Phasen traten erst hervor, als die Granulozytopenie festgestellt wurde.) Familiär nichts zu erwähnen. In jüngeren Jahren gesund. 1924 wegen eines periappendikulären Abszesses im städtischen Mariakrankenhaus. 1926—29 mehrere Male in demselben Krankenhaus wegen einer Osteomyelitis im rechten Oberschenkel und einer im Zusammenhang damit entstandenen Spontanfraktur. 1935 erkrankte er mit allmählich beginnenden Rachenschmerzen, Kopfschmerz und Fieber, weshalb er in 2—3 Wochen zusammen etwa 40 Amidopyrinpulver nahm. 8. 6.—19. 6. 1935 im Mariakrankenhaus gepflegt. Bei der Aufnahme wurde eine Granulozytopenie festgestellt. Leukozyten 1500, Neutrophile 18 %. Das Sternalpunktat war zellenreich. Es war darin eine Vermehrung der myeloischen Prototypen und der Retikulumzellen zu konstatieren. Der Patient wurde mit Campolon behandelt. Die Blutveränderungen verschwanden im Lauf

<sup>1</sup> Über die früheren Schicksale dieses Patienten hat v. BONSdorff berichtet, siehe Finska Läk.s. Handl. 1936, S. 202.

von etwa einer Woche, und der Patient genas. Vor seiner Entlassung aus dem Krankenhaus wurde eine Amidopyrinbelastungsprobe ausgeführt, deren Ergebnis deutlich positiv war. (Nach Einnahme von 0.5 g Amidopyrin sank die Leukozytenzahl in 2 Stunden von 5000 auf 3000.) Späteres Befinden, von kurzdauernden Schnupfeninfektionen abgesehen, gut.

Am 18. 2. 1941 erkrankte der Patient ohne vorausgehende Symptome mit heftigen Rachenschmerzen, Schmerzen in den Gliedern und Fieber.

St. praesens am 22. 2. 1941: Körperbau gewöhnlich. Patient sehr müde, unruhig. Rechtes Knie steif. Im rechten Oberschenkel zahlreiche Narben von Inzisionen und Fisteln. Nervensystem, Lungen, Herz und

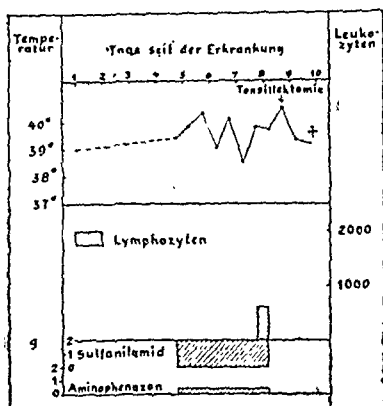


Abb. 11. Fall 25.

Bauch o. B. Harn: Eiw. —. Nyl. —. Fäzes: Wurmeier —. Wagner — Sternalpunkate in Tabelle 14. Blutbilder in Tabelle 15. Zunge etwas belegt. Nur einige kariöse Schneidezähne sind noch vorhanden. Schleimhäute des Mundes, des Zahnfleisches und des Rachens gerötet, trocken. Tonsillen pflaumengross, grubig. Auf der Oberfläche der linken Tonsille reichlich aneinanderstossende graue Beläge. Auch auf der rechten Tonsille etwas Beläge.

Decursus: 25. 2. 1941. Allgemeinzustand verschlechtert. Haut und Sclerae ikterisch. Beläge im Rachen weiter ausgebreitet, links bis zum Gaumen reichend. Vorn unten in der Gingiva ein kleines nekrotisches Gebiet. Im linken Kieferwinkel ein empfindliches taubeneigrosses Drüsenpaket. Harn: Eiw. +. Der Patient hat während 3 Tage 7 g Sulfanilamid und 3.5 g Amidopyrin bekommen.

26. 2. 1941. Tonsillektomie um 14.0 Uhr.

27. 2. 1941. Exitus um 6.30 Uhr.

Obduktionsdiagnose: Tonsillitis haemorrhagica. Oedema glottidis. Pneumoniae lobulares haemorrhagicae pulm. amb. Adhaesiones fibrosae laminar. pleurae l. sin. Bronchitis purulenta. Degeneratio parenchymatosa et adiposa hepatis. Hyperplasia lienis. Nephrosis. Hypertrophia cordis. Icterus universalis.



**Epikrise:** Ein 49jähriger Mann, der früher eine schwere, mehrfach rezidivierende Osteomyelitis und vor 7 Jahren eine nach reichlicher Amidopyrinmedikation entstandene Granulozytopenie-Angina gehabt hat. Eine unmittelbar danach ausgeführte Amidopyrinbelastungsprobe war positiv. Litt wieder an einer nekrotisierenden Angina und nahm vor der Feststellung der Granulozytopenie 3.5 g Amidopyrin und 7 g Sulfanilamid. Eine Tonsillektomie wurde 9 Tage nach der Erkrankung ausgeführt. Starb einen Tag später. Bei der Obduktion wurde ausserdem eine Pneumonie gefunden.

**Fall 26.** (Abb. 12.) 35jährige Arbeiterfrau. Im Epidemiekrankenhaus 4. 6.—20. 6. 1941. (Journ.-Nr. 1353/41.)

**Anamnese:** In der Familie nichts Besonderes. Die Patientin hatte während der letzten 10 Jahre mehrere Anginen. Keine Peritonsillarabszesse. 2—3 mal eine Blasenentzündung, die jedesmal nach mehrwöchiger medikamentöser Therapie heilte. Am 30. 5. 1941 erkrankte die Patientin mit Rachenschmerzen und Fieber, welche Symptome bis zur Ankunft im Krankenhaus fortbestanden.

**St. praesens** am 4. 6. 1941: Körperbau gewöhnlich. Allgemeinzustand ziemlich schlecht. Die Patientin sieht sehr müde aus und ist graublass. Nervensystem, Herz, Lungen und Bauch o. B. Harn: Eiw. —. Nyl. —. Fäzes: Wurmeier —. Wagner —. Sternalpunktate in Tabelle 14. Blutbilder in Tabelle 15. Zunge ziemlich trocken und belegt. Rachen gerötet. Tonsillen weintraubengross, an der Oberfläche rau. Um sie herum eine geringe Anschwellung. Auf der Oberfläche beider Tonsillen etwas heller, dünner Belag. Beiderseits unterhalb des Mandibularwinkels empfindliche Drüsenpakete von der Grösse eines grossen Hühnereies.

**Decursus:** 5. 6. 1941. Allgemeinzustand verschlechtert. Die Beläge im Rachen etwas vermehrt. Es wurde eine Tonsillektomie ausgeführt. Linke Tonsille etwa daumenspitzenlang, rissig, rechte ein wenig kleiner. Die Tonsillen lösen sich leicht ab. Im Innern beider Tonsillen etwas ziemlich dicker gelblicher Eiter.

6. 6. 1941. Allgemeinzustand etwas besser. In den Vorder- und Seitenteilen des Halses eine empfindliche, gerötete Schwellung.

9. 6. 1941. Von Tag zu Tag munterer. Die Rötung und Schwellung am Halse vermehrt, bis in die Mitte des Sternums reichend. Hautfarbe ins Gelbe spielend, Skleren deutlicher ikterisch. In der Basis der linken Lunge halbverhärtetes feuchtes Rasseln. Röntgendurchleuchtung: In der Umgebung der linken Hilusregion eine ziemlich dünne, recht deutlich begrenzte Verschattung. Der Mund bedeutend gereinigt. Oberflächen der Operationswunden fast frei von Belägen.

11. 6. 1941. In der Schwellung am Halse ist eine Fluktuation zu konstatieren. Bei der Punktion bekommt man dicken Eiter, der reichlich

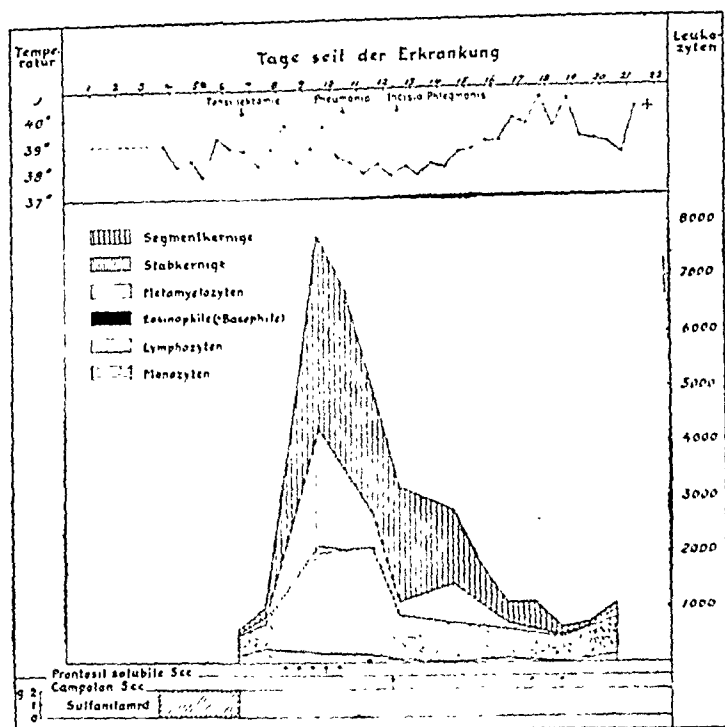


Abb. 12. Fall 26.

Streptokokken enthält. Unmittelbar nach der Punktion wird eine Inzision gemacht, wobei man in eine kleine Eiterhöhle gelangt.

Ein paar Tage später ergab sich, dass sich eine Fistel von der Inzisionswunde zum Ösophagus entwickelt hatte. Der Zustand der Patientin verschlechterte sich allmählich wieder. Um die Fistel begannen sich Nekrosen zu bilden, die sich ausdehnten. Exitus am 20. 6. 1941.

**Epikrise:** Eine 35jährige Frau, die eine rezidivierende Angina gehabt hatte, erkrankte an einer Granulozytopenie-Angina. Eine Tonsillektomie wurde 7 Tage später ausgeführt. Die Blutveränderungen verschwanden schnell. Die Patientin bekam 3 Tage später eine Pneumonie sowie eine sich allmählich entwickelnde, zu einer Ösophagusfistel führende Mediastinitis, wobei die Granulozytopenie rezidierte. Exitus 11 Tage nach der Tonsillektomie.

## b. Ätiologie und Pathogenese.

Aus Tabelle 13 werden die in der Anamnese der Patienten auftretenden, als ätiologische Ursachen der Krise in Betracht kom-

Tabelle 13.

In der Anamnese der Patienten auftretende, als ätiologische Ursachen der Krise in Betracht kommende Faktoren.

Fall	Infektionen	Medikamente	Sonstige ätiologische Faktoren
21	Tonsillitis acuta recidivans	Amidopyrin 7—8 g (vor der Erkrankung im Lauf von 4 Mon.)  Sulfanilamid 5—6 g (nach der Erkrankung im Lauf von 6 T.)	Konstitutionelle häma- topoetische Schwäche
22	Tonsillitis acuta		
23	Tonsillitis acuta recidivans	Neosalvarsan 4,5 g (vor der Erkrankung im Lauf von 3 W.)  Sulfanilamid? (nach der Erkrankung)	
24	Tonsillitis acuta Peritonsillitis	Sulfolpyridin 22 g (vor der Erkrankung im Lauf von 12 T.)  Sulfanilamid 5 g (vor der Erkrankung im Lauf von 5 T.)	
25	Tonsillitis acuta	Amidopyrin 3,5 g (nach der Erkrankung im Lauf von 4 T.)  Sulfanilamid 7 g (nach der Erkrankung im Lauf von 4 T.)	
26	Tonsillitis acuta recidivans		

menden Faktoren ersichtlich: Infektionen, Medikamente und konstitutionelle Faktoren.

Ein infektiös-toxisches Moment trat in allen Fällen deutlich hervor. Sämtliche Patienten hatten eine Tonsillitis im akuten Stadium. Zwei von diesen (22, 24) hatten früher keine Angina gehabt; einer (25) hatte schon einmal früher im Zusammenhang mit einer

Granulozytopenie-Krise eine Angina durchgemacht; drei (21, 23 und 26) hatten eine ausgedehnte, auf eine chronische Tonsillitis hinweisende Anamnese. — Die Patientin 24 hatte unmittelbar vor dem Ausbruch der Krise eine langwierige Respirationsinfektion gehabt.

Verschiedenartige Medikamente waren in vier Fällen zur Verwendung gekommen. Amidopyrin hatte Patientin 21 vor ihrer Erkrankung an den Krisensymptomen 7—8 g im Laufe von 4 Monaten bekommen. In Fall 25 war es während 4 Tagen nach der Erkrankung des Patienten gegeben worden, ehe die wirkliche Natur der Krankheit zutage getreten war. Die Krise, die er 6 Jahre früher gehabt hatte, war aller Wahrscheinlichkeit nach durch Amidopyrin verursacht worden. Die Belastungsprobe gab nämlich sofort danach ein positives Resultat. Sulfanilamid oder Sulfapyridin hatten die Patienten 21, 24 und 25 sowie wahrscheinlich auch Patientin 23 in kleinen Mengen nach der Erkrankung an den Infektionssymptomen während der Zeit vor der Erkennung der Granulozytopenie zu sich genommen. In Fall 23 erkrankte die Patientin an der Krise etwa eine Woche nach dem Abschluss einer kombinierten Neosalvarsan-Wismutkur.

Zeichen einer konstitutionellen Schwäche der Myelopoese waren in Fall 21 festzustellen. In der Familie fanden sich ausser der Granulozytopenie mehrere Fälle von perniziösen und hypochromen Anämien.<sup>1</sup> Bei der Patientin selbst waren die Leukozytenwerte sowohl vor als nach der Krise etwas niedrig. Auf die Respirationsinfektionen nach der Tonsillektomie reagierte sie von neuem mit einer deutlichen Leukopenie.

Aus dem Vorstehenden geht hervor, dass in zwei Fällen (22, 26) von den möglichen ätiologischen Faktoren nur eine Infektion in Frage kam. Bei den anderen Patienten können ausserdem auch andere Faktoren in Betracht kommen. Die durch Amidopyrin sowie durch Neosalvarsan hervorgerufene Granulozytopenie entsteht im allgemeinen unmittelbar nach der Verabreichung des Medikaments, mag es sich um langwierige oder gelegentliche Einnahme handeln (KRACKE und PARKER). In Fall 21 war ihr Anteil unwahrscheinlich, weil die Patientin es nicht unmittelbar vor ihrer Erkrankung gebraucht hatte. Auch in Fall 25 lassen sich diesbezüglich keine sicheren Schlüsse ziehen. Bei dem Patienten

<sup>1</sup> Die Familie der Patientin werde ich später in einer besonderen Veröffentlichung eingehender zu behandeln.

war die vorhergehende Krise zwar wahrscheinlich durch Amidopyrin ausgelöst worden. Das klinische Krankheitsbild, besonders das Auftreten von Nekrosen, spricht jedoch dafür, dass er diesmal schon vor der Verwendung des Amidopyrins eine Granulozytopenie hatte. Die Erfahrungen mit Sulfanilamidpräparaten zeigen, dass die Granulozytopenie im allgemeinen nach langdauernder und reichlicher Anwendung entsteht, sofern der Patient diese Präparate nicht schon früher bekommen hat. Hat er sie früher erhalten, so kann sich eine Sensibilisierung entwickeln, so dass die Granulozytopenie schon nach kurzdauernder und geringer Anwendung ausbrechen kann (DOMAGK und HEGLER). In meinen Fällen wurde von der Sulfanilamidmedikation im Zusammenhang mit der Tonsillektomie Gebrauch gemacht, und das Blutbild zeigte während dieser medikamentösen Therapie eine schnelle Regeneration. Hiernach zu schliessen, dürfte sie in der Ätiologie der Krise keine nennenswerte Rolle spielen. Mit Sicherheit lässt sich nicht entscheiden, welchen pathogenetischen Anteil die verschiedenen Faktoren in den betreffenden vier Fällen gehabt haben. Wie bei Patientin 21, bei der sowohl der infektiöse als der konstitutionelle Faktor auftrat, kann auch bei den anderen eine kombinierte Ätiologie in Frage kommen. Aus diesem Grunde ist auch nicht sicher auszumachen, ob die Angina in diesen Fällen primärer oder sekundärer Natur war.

### c. Das klinische Krankheitsbild.

Der gleichzeitig mit der Granulozytopenie-Krise auftretenden Tonsillitis wie auch den anderen Entzündungsprozessen ist es eigentümlich, dass sie leicht einen nekrotisierenden Charakter annehmen. In zwei (21 und 24) meiner Fälle fanden sich in den Tonsillen keine makroskopischen Nekrosen, aber bei Patientin 21 zeigten sie sich bei der mikroskopischen Untersuchung. Bei den Patienten 22, 23 und 26 waren sie unbedeutend. Bei den zwei ersteren wurden sie nur in der Umgebung einer Inzision, die wegen Verdachts auf einen Peritonsillarabszess gemacht worden war, also nur im beschädigten Gewebe, angetroffen. Fall 25 war der einzige, in dem die Nekrosen einen grossen Umfang angenommen hatten.

Ausser einer Tonsillitis wurden bei den Patienten auch andere entzündliche Krankheiten festgestellt. In Fall 21 ergab sich eine

Bronchitis. Patientin 24 hatte den Folgezustand einer langwierigen Pneumonie und dazu eine hartnäckige Enteritis. Bei Patient 25 wurden erst bei der Obduktion in beiden Lungen Pneumonieherde festgestellt. In Fall 26 entwickelte sich desgleichen eine Pneumonie und dazu noch eine Mediastinitis, Komplikationen, die kurze Zeit nach der Tonsillektomie entdeckt wurden.

Die klinischen Befunde waren somit die, die typisch im Zusammenhang mit der Krise auftreten. In bezug auf die Tonsillitis stellte die Spärlichkeit der Nekrosen eine Besonderheit dar. Sie kamen in geringerer Menge vor, als es in Verbindung mit der Krise bei den sich sekundär entwickelnden Anginen der Fall ist. Das würde darauf hinweisen, dass die Tonsillitisinfektion bei meinen Patienten hauptsächlich primär war, ein Verhalten, für das auch die anamnestischen Befunde sprechen. In den Fällen 22 und 23 fand sich stark ausgeprägt ein peritonsilläres Ödem, das Verdacht auf einen Abszess erregte und zu einer Inzision veranlasste. Ein solches Ödem ist bei der Granulozytopenie-Angina nicht selten (ZANGE).

#### d. Die hämatologischen Befunde.

Die Sternalpunktate (Tabelle 14) vertraten in meinen Fällen verschiedene Typen. Am stärksten waren die Veränderungen in Fall 25. Das Punktat zeigte eine totale myeloische Aplasie. Die einzigen myeloischen Zellen, die angetroffen wurden, waren Myeloblasten. Die Menge der Lymphozyten und Retikulumzellen war bedeutend vermehrt. In den Fällen 24 und 26 waren die Veränderungen ähnliche: das Mark war promyelozytär, nicht sehr zellenarm. Die Menge der Lymphozyten und Retikulumzellen war auch hier vermehrt. Typisch war für diese Punktate ferner, dass die in ihnen angetroffenen myeloischen Zellen deutliche pathologische Veränderungen erkennen liessen: die Kerne der Zellen entsprachen einem älteren Reifestadium, als das Plasma und die Granulation voraussetzten. So war das Plasma der wenigen festgestellten Myelozyten und Metamyelozyten im allgemeinen basophil und die Granulation bemerkenswert grob. In Fall 23 zeigte das Sternalpunktat auch Veränderungen der oben beschriebenen Art, aber in leichterer Ausprägung. Noch geringfügiger waren die Ab-

Tabelle 14.  
Die Sternalpunkte der Granulozytopenie-Fälle.

Nr.	Datum der Tonsillek- tomie	Datum	%										Zahl auf 400 L						Retikulum- & Plasmaz.	Megakar.	Normobl.	Makrobl.	Proerythrobl.	Rote Mitosen	Weisse Mitosen			
			Bas. Myeloz. & Leukoz.	Eos. Myeloz.	Eos. Leukoz.	Myelobl.	Neutr. Promyeloz.	Neutr. Myeloz.	Neutr. Metamyeloz.	Neutr. stabk. Leukoz.	Neutr. segm. Leukoz.	Lymphoz.	Monoz.	9	0	60	4	0								1	1	
21	31/5-38	11/6	0.25	0	0	1.0	9.25	20.0	26.25	24.0	3.75	14.75	0.75	9	0	60	4	0	1	1								
22	16/12-38	16/12	0.25	6.25	1.75	2.0	9.0	13.0	26.25	11.0	5.25	23.0	1.25	15	0	59	6	0	1	0								
		18/1	0	3.50	1.25	1.25	2.25	13.0	19.50	21.0	10.50	26.0	1.75	6	0	97	2	0	0	0								
		14/2	0	3.25	0.50	1.25	4.25	12.25	18.75	12.0	17.0	28.0	2.75	14	0	100	5	1	0	3								
23	21/12-38	21/12	0	4.75	1.25	2.0	43.75	15.0	7.75	2.25	0.25	16.25	6.75	18	0	67	5	0	0	2								
		29/12	0	2.50	0.75	1.25	6.50	20.50	21.75	25.0	14.0	7.25	0.50	13	0	37	3	0	1	0								
24	19/8-39	19/8	0.25	0	0	2.25	39.50	8.50	2.75	0.50	0	46.25	0	13	0	21	7	1	1	0								
		25/8	0.25	0	0	0.50	2.50	23.50	37.50	22.75	7.25	5.75	0	8	0	14	0	0	1	0								
25 <sup>1</sup>	26/2-41	26/2	0	0	0	2.68	0	0	0	0	0	42.68	0	35.76 %	1.22 %	16.52 %	0.38 %	0.38 %	0.38 %									
26	5/6-41	5/6	0	0	0	5.0	19.50	4.25	0.75	1.25	0	68.0	2.25	26	2	102	10	1	2	4								

Die Sternalpunkte der Ordinalzahl 1

Die Sternalpunkte sind alle Zellarten in Prozent angegeben.

<sup>1</sup> Wegen des geringen Zellgehalts des Punktes sind alle Zellarten in Prozent angegeben.

weichungen im Vergleich zu den obigen bei Patient 22, bei dem nur die Menge der fertigen Neutrophilen im Marke bedeutend herabgesetzt war, aber die der myeloischen Prototypen nicht nennenswert von der Norm abwich. In Fall 21 war vor der Tonsillektomie kein Sternalpunktat entnommen worden.

In den Blutbildern (Tabelle 15) vertraten die Veränderungen ebenfalls verschiedene Stadien. Bei Patient 25 betrug die Menge der weissen Zellen nur 600, und sie waren sämtlich Lymphozyten. In den Fällen 21, 21 und 26 trat ausser einer starken Neutro- und Leukopenie eine Aneosinophilie auf. Monozyten fanden sich bei allen drei Patienten, doch war ihre absolute Zahl merkbar niedriger als normalerweise. In Fall 23 waren die Veränderungen relativ leichte; die Gesamtzahl der Leukozyten lag an der unteren Grenze des Normalen, es bestand keine Aneosinophilie, und ausserdem war eine Monozytose (auch absolut) festzustellen. Eine von den anderen abweichende Reaktion des Blutes zeigte sich in Fall 22. Es handelte sich um eine schnell zunehmende Leukozytose. Die Zahl der Neutrophilen war niedrig und die der Lymphozyten sowie der Monozyten bedeutend erhöht.

Die beschriebenen hämatologischen Veränderungen sind ähnlicher Art, wie sie früher im Schrifttum angegeben worden sind. ROHR teilt die bei der Granulozytopenie-Krise vorkommenden Knochenmarksveränderungen in drei Haupttypen, zwischen denen zahlreiche Übergangsformen auftreten: 1) Totale Aplasie der Leukopoese, wobei die Menge der lymphoiden und der Retikulumelemente meist bedeutend vermehrt ist. Die Schädigung der Leukopoese ist in diesen Fällen so schwer, dass eine Regeneration im allgemeinen nicht mehr möglich ist. 2) Das promyelozytär-myelozytäre Mark, wie es in klinisch schweren Fällen anzutreffen ist. 3) Knochenmark, in dem nur die fertigen Zellen (Segmentkernige und Stäbe) fehlen. Ein solches Knochenmarksbild zeigt sich in leichten Fällen. In meinen eigenen Fällen entsprach der Knochenmarksbefund des Patienten 25 durchaus dem ersten Typus ROHR's, das Knochenmarksbild der Patienten 24, 26 und auch 23 gehört am ehesten zu der zweiten und das von Patient 22 zu der dritten Gruppe. Ausser nach dem Sternalpunktat können wir auch nach dem Blutbild den Schweregrad der Krise beurteilen. Wenn die Gesamtzahl der Leukozyten im Blute 1000 unterschreitet und wenn ausser den Neutrophilen und Eosinophilen auch die Monozyten



Tabelle 15.  
Die Blutbilder der Granulozytopenie-Fälle<sup>1</sup>.

Nr.	Datum der Ton- silek- tomie	Datum	Hb	Erythroz.	Index	Leuko.	Neutroph.	Myeloz.	Meta- myeloz.	Stab- kernige	Segment- kernige	Basoph.	Eosinoph.	Monoz.	Lymphoz.
21	31/5 — 38	14/5 — 38	70	4336	0.81	3000									
		30/5 »	66	4600	0.72	500	12.0	0	2.0	3.0	7.0	0	0	7.0	81.0
		1/6 »				550	23.0	0.5	2.0	8.0	12.5	0	0	6.5	70.5
		2/6 »	64	3860	0.84	650	41.0	2.0	3.0	8.0	28.0	0	0	5.0	54.0
		3/6 »				950	30.5	0	1.0	6.0	23.5	0	0	4.0	65.5
		4/6 »	66	4130	0.80	1850	47.0	0	0	4.5	42.5	0	0	2.5	50.5
		6/6 »	65	4580	0.71	2200	59.0	0	0	4.0	55.0	0	0	2.0	39.0
		7/6 »				2625	43.5	0	0	4.0	39.5	0	0	4.0	52.5
		8/6 »	68	4400	0.77	2350	31.0	0	0	4.5	26.5	0	0	4.0	65.0
		9/6 »	65/70	4500	0.78	2400	46.0	0	0	3.0	43.0	0	0	6.0	48.0
		11/6 »	68/73	4860	0.74	2800	46.0	0	0	4.0	42.0	0	0	5.0	49.0
		13/6 »				2900	40.5	0	0	3.5	37.0	0	0	9.0	49.0
		15/6 »	72/78	4900	0.80	3400	53.5	0	0	5.0	48.5	0	0	8.0	38.5
		17/6 »	70/76	4730	0.80	3300	58.0	0	0	3.0	55.0	0	0	7.0	35.0
		21/7 »	69/75	4620	0.81	3600	68.0	0	0	2.0	66.0	0	0	5.0	27.0
		13/9 »	80/86	4490	0.96	3050	59.0	0	0	3.0	56.0	4.5	1.0	6.5	29.0
		6/2 — 39	65/70	4296	0.81	3500	68.5	0	0	1.0	67.5	0	0	8.0	23.5
		24/5 »	68/73	4656	0.78	3000	57.0	0	0	2.0	55.0	0	0	9.0	34.0
		22/6 »	64/69	3904	0.88	4500	71.0	0	0	3.0	68.0	0	0	11.0	18.0
		2/11 »	66/74			3800	71.0	0	5.0	38.0	28.0	0	0	3.0	26.0
		4/11 »	64/72	4392	0.81	2600	79.0	0	5.0	40.0	34.0	0	1.0	9.0	11.0
		8/11 »	62/70	4696	0.74	2900	62.0	0	4.0	24.5	33.5	0	0.5	13.5	24.0
		28/5 — 40	62/67	4008	0.84	6000	61.5	0	0	3.0	58.5	0	0	10.0	28.5
		18/10 »	65/70	4000	0.88	5000	61.5	0	0	2.5	59.0	0	0.5	10.0	28.0
22	16/12 — 38	14/12 — 38	80/93	4996	0.93	17100	24.5	0	0	2.5	22.0	0.5	0	12.0	63.0
		16/12 »	81	5340	0.75	35200	32.0	0	0	5.0	27.0	1.5	0.5	8.5	57.5
		17/12 »	81	5000	0.81	32200	29.5	0	0	3.5	26.0	1.5	0	15.0	54.0
		19/12 »	83	4960	0.84	11800	43.0	0	0	6.0	37.0	1.0	1.0	7.0	48.0
		18/1 — 39	78/84	4380	0.96	7900	24.0	0	0	1.5	22.5	0.5	1.0	4.5	70.0
		14/2 »	72/78	4400	0.89	7400	32.0	0	0	1.0	31.0	1.0	1.0	6.0	60.0
23	21/12 — 38	19/12 — 38	66/77	4196	0.91	3500	3.5	0	0	0	3.5	0.5	11.0	64.0	21.0
		21/12 »	65	3700	0.88	5000	14.0	0	2.0	8.5	3.5	1.5	8.5	42.0	34.0
		22/12 »	64	3600	0.89	8200	46.5	0	16.0	20.0	10.5	2.0	4.0	26.5	21.0
		24/12 »	65	3720	0.87	7000	54.0	0	2.0	17.0	35.0	1.0	6.0	11.0	28.0
		27/12 »	65	3600	0.90	5200	56.5	0	1.0	13.0	42.5	1.5	7.0	11.5	23.5
		29/12 »	66	4000	0.83	5400	65.0	0	0	5.0	60.0	1.0	3.0	5.5	25.5

<sup>1</sup> Die Ursache zu der Verschiedenheit der in dieser wie in einigen folgenden Tabellen auftretenden unkorrigierten Hämoglobinwerte ist im Zusammenhang mit der Methodik, Seite 13, angegeben.

Tabelle 15 (Forts.).  
Die Blutbilder der Granulozytopenie-Fälle.

Nr.	Datum der Ton- siftek- tonie	Datum	Hb	Erythroz.	Index	Leuko- z.	Neutroph.	Myelo- z.	Meta- myelo- z.	Stab- kernige	Segment- kernige	Basoph.	Eosinoph.	Monoz.	Lymphoz.
24	19/8 —39	14/3 —35	66			15100	62.5	0	0	4.0	58.5	0.5	3.5	3.5	30.0
		11/8 —39	55	3540	0.78	4600	44.0	0	0	14.0	30.0	0.1	0	4.6	51.0
		17/8 „	60	3490	0.86	1850	2.0	0	0	2.0	0	2.0	0	5.5	90.5
		18/8 „	58	3380	0.86	2250	1.0	0	0	1.0	0	1.5	0	4.5	93.0
		19/8 „	61	3440	0.89	2150	4.5	0	0.5	2.0	2.0	1.0	0	8.5	86.0
		20/8 „	59	3400	0.87	3220	31.0	3.4	3.6	12.2	11.8	0.6	0	8.2	60.2
		(am M.)													
		20/8 —39	59	3390	0.87	5300	54.6	10.0	5.3	11.3	18.0	0	0	10.7	44.7
		(am A.)													
		21/8 —39	61	3510	0.87	7550	53.4	5.0	4.8	23.6	20.0	0.2	0	6.6	39.8
		22/8 „	63	3630	0.87	8960	67.2	5.4	7.4	28.0	26.4	0	0	3.2	29.6
		23/8 „	65	3700	0.88	11400	67.4	3.6	3.4	19.2	41.2	0	0	3.0	29.6
		24/8 „	66	3710	0.89	12100	63.6	3.2	6.8	19.8	43.8	0	0	3.0	23.4
		25/8 „	68	3820	0.89	11800	71.6	2.0	9.0	17.6	13.0	0	0	2.4	26.0
		28/8 „	73	4060	0.90	7800	54.6	0	1.6	5.8	47.2	0.2	0.2	2.0	43.0
		30/8 „	79	4500	0.88	7900	68.8	0	2.4	5.2	61.2	0.6	0	2.6	28.0
		12/9 „	74	4100	0.90	7600	48.4	0	0	1.8	46.6	1.0	6.0	4.6	40.0
25	26/2 —41	26/2 —41	75/87	4070	1.06	600	0	0	0	0	0	0	0	0	100.0
26	5/6 —41	5/6 —41	86/100	4844	1.03	600	9.5	0	0	4.0	5.5	0	0	24.0	66.5
		6/6 „	80/93	4561	1.01	1000	29.0	0	3.5	10.5	15.0	0	0	25.0	46.0
		7/6 „				5200									
		8/6 „	77/87	4340	1.02	7800	74.0	0	1.0	27.5	45.5	0	0	2.5	23.5
		9/6 „				6800									
		10/6 „				5000	58.0	0	0	13.0	45.0	0	0	3.0	39.0
		11/6 „	69/80	1144	0.96	3200	75.0	0	0	8.0	67.0	0	0	1.0	24.0
		13/6 „	67/78	3761	1.03	2800	74.5	0	0	26.5	48.0	0	0	0.5	25.0
		14/6 „				1800									
		15/6 „	64/74	4032	0.91	1100	44.0	0	0	10.0	34.0	0	0	7.0	49.0
		16/6 „				1100									
		17/6 „	62/72	3568	1.0	630	26.0	0	0	6.0	20.0	0	0	2.0	72.0
		18/6 „				700									
		19/6 „	62/72	3656	0.98	1070	23.0	0	0	15.0	8.0	0	0	12.0	65.0

fehlen oder ihre Zahl bedeutend herabgesetzt ist, muss die Prognose als unsicher gelten (ROHR). Solche schwere Veränderungen kamen bei den Patienten 21, 24, 25 und 26 vor. Die Monozytose fassen mehrere Forscher als ein günstiges Symptom auf (BOCK, LICHTENSTEIN, SCHULTEN, STRASSER). Ferner betrachtet es SCHULTEN als ein gutes Zeichen, dass sich keine Aneosinophilie ausbildet. In meinem Fall 23 lagen die erwähnten prognostisch günstigen Symptome vor. Ähnliche Blutreaktionen, wie sie bei den Patienten 22 und 23 vorkamen, können auch im Zusammenhang mit der Mononucleosis infectiosa auftreten. Bei diesen Patienten fehlten jedoch die gewöhnlichsten klinischen Symptome dieser Krankheit: die allgemeine Drüsenschwellung und die Vergrößerung der Milz. Ausserdem traten im Knochenmark beider für Granulozytopenie typische Veränderungen auf, die nicht bei Mononucleosis infectiosa anzutreffen sind. Diese Umstände sprechen, wie mir scheint, dafür, dass es sich um Granulozytopenie handelte. Eine neutropenische Leukozytose, wie in Fall 22, ist im Heilungsstadium der Granulozytopenie-Krise wohlbekannt. Dabei kann man sogar leukämieähnliche Blutbilder finden. Ich sehe somit in diesem Fall eine Granulozytopenie-Angina, die erst im Heilungsstadium zur Beobachtung und Behandlung kam.

Unter Berücksichtigung der oben beschriebenen, im hämatologischen Status angetroffenen Veränderungen und des klinischen Krankheitsbildes habe ich den Schweregrad der Granulozytopenie-Krise der Patienten so analysiert, wie er in Tabelle 6 auftritt.

#### e. Die Wirkung der Tonsillektomie.

Die Tonsillektomie wurde innerhalb 3—12 Tage nach dem Ausbruch der Symptome ausgeführt. In Fall 25 traten im Zustand des Patienten nach der (8 Tage nach dem Ausbruch der Krankheit ausgeführten) Operation keinerlei Veränderungen nach dem Besseren hin ein. Der ausserordentlich schwere septische Zustand blieb bestehen, und der Patient starb schon einen Tag nach dem Eingriff. Es handelte sich denn auch um einen sowohl klinisch als hämatologisch als infaust zu betrachtenden Fall. Im Zustand der anderen fünf Patienten war dagegen unmittelbar nach der Tonsillektomie ein deutlicher Umschwung festzustellen. Der schlechte All-

gemeinzustand besserte sich schnell. In den Fällen 21—24 verschwand das Fieber in 2—3 Tagen. In Fall 26, bei dem die Tonsillektomie 6 Tage nach der Erkrankung stattfand, war die Besserung des Allgemeinzustands nur vorübergehender Art, und es trat danach kein Fieberabfall ein. Drei Tage später klärte sich denn auch die Ursache zu dem Fortbestehen des Fiebers auf. Es war bei der Patientin eine Pneumonie zu konstatieren, und überdies entwickelte sich bei ihr allmählich eine zu einer Ösophagusfistel führende schwere nekrotische Mediastinitis. Sie starb 11 Tage nach der Operation. — Besondere Aufmerksamkeit wurde dem Zustand der Operationswunden und der postoperativen Entwicklung zugewandt. Die Beläge auf den Operationswunden waren keineswegs grösser als gewöhnlich, und die Wunden heilten, auch in Fall 26, in kurzer Zeit normal.

Hand in Hand mit der klinischen Besserung verschwanden auch die peripheren Blutveränderungen schnell. In Fall 21 ist am dritten Tag nach der Operation ein deutlicher Anstieg der Leukozytenzahl zu bemerken. Noch schneller war die Regeneration in den Fällen 23, 24 und 26, besonders in den beiden letzteren. Bei Patientin 24 erhöhte sich die Zahl der Leukozyten in 2 Tagen um 5400 und bei Patientin 26 in derselben Zeit um 4600. Bei allen drei Patientinnen zeigte sich zugleich eine beträchtliche Linksverschiebung der Neutrophilen, eine Erscheinung, die im Regenerationsstadium der Krise gewöhnlich ist. In Fall 22, bei dem vor der Operation eine neutropenische starke Leukozytose auftrat, verschwanden die Blutveränderungen ebenso im Verlauf von 3 Tagen.

Mustert man die Sternalpunktate nach der Operation durch, so findet man auch in ihnen ein Verschwinden der vor dem Eingriff bestehenden Veränderungen. Doch scheint es verhältnismässig lange zu dauern, bis die Zahl der fertigen Neutrophilen die normale Höhe erreicht (Fall 21 und 22).

Bei der Beurteilung des Effektes der Tonsillektomie auf die Patienten ist zu beachten, dass ausser der Operation auch andere Therapie zur Verwendung kam, abgesehen von dem leichten Fall 23 (und Fall 25, in dem der Zustand präagonal war), dem gegenüber therapeutische Massnahmen als nutzlos betrachtet wurden. In den anderen Fällen wagte man wegen des Ernstes der Situation sich nicht ausschliesslich auf die Tonsillektomie zu verlassen.

Tabelle 16.

Ausser der Tonsillektomie medikamentöse und andere Behandlung.

Fall	L e b e r	Sulfanilpräparate	Sonstige Therapie
21	Campolon zusammen 5 cm <sup>3</sup> × 18 im Lauf von 8 T.	Prontosil solub. zusammen 5 cm <sup>3</sup> × 3 im Lauf von 2 T.	—
22	—	Sulfanilamid zusammen 3 g im Lauf von 3 T.	—
23	—	—	—
24	Campolon zusammen 5 cm <sup>3</sup> × 3 im Lauf von 2 T.	Prontosil solub. zusammen 5 cm <sup>3</sup> × 5 im Lauf von 5 T.	Bluttransfusion
25	—	—	—
26	Campolon zusammen 5 cm <sup>3</sup> × 3 im Lauf von 7 T.	Prontosil solub. zusammen 5 cm <sup>3</sup> × 5 im Lauf von 3 T. Sulfanilamid zusammen 6 g im Lauf von 6 T.	—

Die anderen Behandlungsmassnahmen wurden weggelassen, sobald im Zustand der Patienten eine Wendung zum Besseren eingetreten war. Infolgedessen und da die Patienten in verschiedenen Krankenhäusern gepflegt wurden, war die ausser der Tonsillektomie angewandte Therapie in keiner Weise systematisch. Die Behandlungsmassnahmen werden aus Tabelle 16 ersichtlich. Ausser Sulfanilamid und Campolon wurden keine anderen Medikamente gebraucht. Ersteres wurde allen in Rede stehenden 4 Patienten in kleinen Mengen verabreicht. Vor der Operation hatten es 5 Patienten (21—25) bekommen, ohne dass die Entwicklung der Krise dadurch verhindert wurde. Campolon wurde den Patientinnen 21, 24 und 26 gegeben, energisch nur in dem ersten Fall. Die Erfahrungen mit Lebertherapie bei der Granulozytopenie zeigen, dass sie in manchen Fällen Nutzen stiften kann, aber durchaus nicht immer wirksam ist. Ihre Wirkung ist nicht schnell (s. v. BONSDORFF). Es bleibt also von den Massnahmen, die eine günstige schnelle Wendung im Verlauf der Krise bei meinen 5 Pati-

enten herbeiführen konnten, am ehesten die Tonsillektomie übrig. Die ausser ihr angewandten therapeutischen Massnahmen machen jedoch die exakte Beurteilung des Effektes der Operation unmöglich. Wenn ich von einer Wirkung der Tonsillektomie bei meinen Patienten spreche, geschieht es mit dem Vorbehalt, dass der eventuelle Anteil der anderen Therapie nicht ganz ausgeschlossen werden kann.

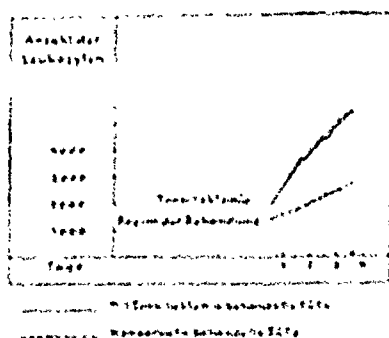


Abb. 17. Mittelwerte der Leukozyten in 5 operierten und 6 konservativ behandelten Fällen.

Ferner ist noch die Möglichkeit einer spontanen Heilung in Betracht zu ziehen. Diese kommt in Frage besonders in Fall 22, der erst im Heilungsstadium zur Behandlung kam, und in Fall 23, bei dem die Symptome der Krise leicht waren. Doch war auch in diesen Fällen, wie in den drei anderen, unmittelbar nach der Tonsillektomie eine deutliche Wendung im Krankheitsverlauf festzustellen. Gegen eine spontane Heilung spricht auch das schnelle Verschwinden der Blutveränderungen. Obgleich die spontane oder bei nicht-operativer Therapie eintretende Regeneration schnell vor sich gehen kann (Gortz), habe ich nach Kenntnisnahme der Literatur die Auffassung gewonnen, dass das Verschwinden der Veränderungen in so schnellem Tempo, wie bei meinen eigenen Fällen, nicht gewöhnlich ist. Dieser Auffassung verleiht der in Abbildung 17 ausgeführte Vergleich eine Stütze. In der erwähnten Abbildung habe ich die nach der Tonsillektomie auftretenden Mittelwerte der Leukozyten bei meinen Fällen (mit Ausschluss von Fall 25) und bei insgesamt 6 von AARNE und v. BOSSBOM in Finnland veröffentlichten mit konservativen Methoden behandelten Fällen berechnet. Hierbei zeigte sich, dass die durch-

schnittliche Vermehrung der Leukozyten bei meinen Patienten schneller als in dem Vergleichsmaterial erfolgte.

#### f. Besprechung der Ergebnisse.

Meine Fälle lassen erkennen, dass das Infektionsmoment in der Pathogenese der Granulocytopenie-Angina eine wichtige Rolle spielt. Sie bringen weiteres Licht in die Frage, ob man mittels der Tonsillektomie auf diesen ätiologischen Faktor und dadurch auf den Verlauf der Krise einwirken kann. Der Effekt war in 5 meiner Fälle unschädlich, in 4 (21—24) ausserdem offenbar günstig. In einem Fall (26) war der Nutzen fraglich, und in einem (25) wirkte sich die Tonsillektomie nachteilig aus.

Wie bereits erwähnt (S. 28), ist die Tonsillektomie als kontraindiziert betrachtet worden, weil sie eine Ausbreitung der Nekrosen und eine Zunahme der Sepsisgefahr verursachen soll. Die Heilung der Operationswunden war jedoch in meinen Fällen völlig normal bei 5 Patienten, die in dieser Beziehung mein Beobachtungsmaterial bilden. (In Fall 25 lassen sich aus der Heilung der Wunden keine Schlüsse ziehen, da der Patient kurz nach der Operation starb.) Bei Durchsicht der anderen in Tabelle 6 wiedergegebenen Fälle zeigt sich, dass die Nekrosen, die bei einem Patienten von GRAHE und KINDLER und bei den beiden Patienten von LINCK schon früher vorhanden waren, sich nach der Operation ausbreiteten oder dass sich solche in den Wundflächen entwickelten. In den anderen Fällen der Tabelle werden Angaben darüber entweder vermisst oder die Patienten starben kurz nach der Operation. In den Fällen, bei denen Nekrosen entstanden, trat im Blute keine schnelle Regeneration ein. Bei meiner Patientin 26 entwickelte sich nach der Tonsillektomie parallel mit der Zunahme der Granulocytopenie eine nekrotisierende Entzündung, obgleich nicht im Rachen, in dem die Operationswunden damals schon beinahe geheilt waren. Die Tendenz zur Entwicklung von Nekrosen scheint also von dem hämatologischen Zustand abhängig zu sein. Diese operierten Fälle bestätigen die klinische Erfahrung, dass die

Nekrosen im Regenerationsstadium der Krise schnell verschwinden, wenn die Menge der Granulozyten sich im Blute erhöht (SCHULZES, Rom). Auch schwere granulozytopenische Blutveränderungen stellen somit an sich keine Gegenanzeige gegen eine Tonsillektomie dar, und es besteht keine Gefahr einer Ausbreitung der Nekrosen, wenn man durch die Tonsillektomie eine genügende Erhöhung der Granulozyten erreicht.

ZANGL betrachtet, wie erwähnt, die Tonsillektomie als indiziert in Fällen, bei denen die Tonsillitisinfektion primär ist, dagegen aber nicht bei der sekundären Granulozytopenie-Angina. Wie er aber selbst hervorhebt, ist die Auseinanderhaltung dieser verschiedenen Anginaformen in der Praxis schwierig und oftmals unmöglich. Das ergibt sich deutlich, von meinen eigenen Patienten abgesehen, auch aus den anderen Fällen in Tabelle 6. Auch bei den meisten von diesen können ausser dem Infektionsmoment Medikamente und andere pathogenetische Faktoren in Frage kommen. Selbst wenn die Blutkrise nicht ausschliesslich infolge der Tonsillitis zustande gekommen sein sollte, sondern durch das Zusammenwirken der Tonsillitis und anderer Faktoren oder lediglich aus anderen Ursachen, kann das Endergebnis bei den Granulozytopenie-Anginen doch dasselbe und zwar eine schwere toxisch-septische Tonsillitis sein. Im Hinblick hierauf besitzt die obige Differenzierung zwischen primärer und sekundärer Tonsillitis meiner Ansicht nach nur theoretische Bedeutung, die bei der Indikationsstellung der Tonsillektomie nicht unbedingt in Betracht gezogen zu werden braucht.

Von Wichtigkeit ist dagegen die genaue Aufklärung des klinischen und hämatologischen Zustands des Patienten. Die klinische Erfahrung lehrt, dass die Pneumonie, die sich bei Granulozytopenie entwickelt, fast hundertprozentig zum Exitus führt. Aus diesem Grunde ist die Tonsillektomie bei dieser Komplikation kontraindiziert. In meinem Fall 25 wurde die Pneumonie nicht klinisch festgestellt, sondern erst bei der Obduktion aufgedeckt. Dieser Fall ebenso wie die Ergebnisse anderer Forscher (GRAHE) zeigen, dass eine Operation keinen Zweck hat und dass sie in Fällen kontraindiziert ist, in denen ein weitentwickelter septischer Zustand vorliegt.



Das entspricht durchaus der Auffassung, die im allgemeinen auf otologischer Seite bezüglich der tonsillo-genen Sepsiden herrscht.

Die hämatologische Untersuchung ist auch von hervorragender Bedeutung, wenn es gilt, die Fälle in Hinsicht auf die Prognose und die Therapie zu beurteilen. Da wir die Heilungsmöglichkeiten noch nicht auf Grund des Bluthildes einschätzen können, ist die Untersuchung des Sternalpunktates unentbehrlich. Dabei bleiben einem solche Erfahrungen wie in meinem Fall 25 erspart. Der Patient wurde unmittelbar nach der Sternalpunktion vor der Analyse des Punktates operiert. Wie erwähnt, ergab sich bei dieser eine totale myeloische Aplasie. Die Operation war mithin in diesem Fall sowohl klinisch als auch hämatologisch kontraindiziert, was sich bedauerlicherweise zu spät herausstellte.

Es ist auch ersichtlich geworden, dass die Tonsillektomie nicht immer von Erfolg begleitet ist, trotzdem ausser der Angina keine anderen entzündlichen Lokalisationen bestanden und selbst wenn eine Heilung auch hämatologisch möglich sein sollte (mein Fall 26, die Fälle von LINCK und WESTERGREN). In solchen Fällen ist die Operation keine hinreichende therapeutische Massnahme. Dazu müssen eine die Myelopoese stimulierende Behandlung und die Verabreichung von Sulfanilamidpräparaten hinzugefügt werden. Die Erfahrungen mit der letzteren Therapie bei den Granulozytopenie-Anginen sind beschränkt, aber sie zeigen, dass man mit ihrer Hilfe überraschend gute Erfolge erzielen kann (IVES, MENDES DE LEON). Möglicherweise können durch Anwendung von Sulfanilamid im Zusammenhang mit der Operation die ausschliesslich mit der Tonsillektomie erreichten Resultate verbessert werden.

Hinsichtlich der eventuellen Gefahren der Operation habe ich nicht genügend Erfahrungen. Die Wirkung der bei der Tonsillektomie angewandten Lokalanästhesie erstreckt sich teilweise bis auf den Larynx. Ob die in meinem Fall 26 sowie in den beiden Fällen von GRAHE und WESTERGREN nach der Tonsillektomie entwickelte Pneumonie auf einer in dieser Weise erhöhten Aspirationsgefahr beruht hat, bleibt unentschieden. Jedenfalls nehmen die mit der Tonsillektomie verknüpften Gefahren um so mehr zu, je weiter die Operation hinausgeschoben wird.

Meine bisherigen Ergebnisse mit der Tonsillektomie bei der Gra-

nulozytopenie-Angina sind eher positiv als negativ. Der Eingriff verdient mehr Beachtung, als ihm bisher im Schrifttum zuteil geworden ist.

#### g) Zusammenfassung.

Es werden 6 Fälle von Angina granulocytopenica angeführt, bei denen während der Krise eine Tonsillektomie ausgeführt wurde.

Fall 21. Klinisch und hämatologisch schwere Krise. Die Tonsillektomie wurde 6 Tage nach dem Beginn der Krise ausgeführt. Die klinische Heilung erfolgte schnell. Die hämatologische Regeneration begann 3 Tage nach der Operation und verlief danach schnell. Allerdings bekam die Patientin auch Leberinjektionen. Nach Ansicht des Verf. trug die Operation entscheidend zur Heilung bei.

Fall 22. Patient mit akuter Tonsillitis, dessen Blutbild eine starke neutropenische Leukozytose zeigle. Heilte klinisch und hämatologisch schnell nach der Tonsillektomie, die 10 Tage nach der Erkrankung ausgeführt wurde. Der Verf. betrachtet den Fall als eine Granulozytopenie-Angina, die erst im Heilungsstadium zur Beobachtung und Behandlung kam und die wahrscheinlich auch ohne Operation geheilt wäre.

Fall 23. Leichter Fall, bei dem das Blutbild ausser Leuko- und Neutropenie Monozytose aufwies. Die Tonsillektomie wurde 12 Tage nach der Erkrankung ausgeführt, und danach erfolgte die Heilung schnell. Auch in diesem Fall hält der Verf. die Heilung ohne Tonsillektomie für wahrscheinlich.

Fall 24. Bei der Patientin entwickelte sich eine schwere Granulozytopenie-Angina im Heilungsstadium einer langwierigen mit Sulapyridin behandelten Pneumonie. Drei Tage nach ihrem Ausbruch erfolgte nach der Tonsillektomie ausserordentlich schnell eine Regeneration des Blutes und eine klinische Heilung. Die Patientin bekam auch kleine Mengen Campolon und Protosil. Heilung nach Ansicht des Verf. ohne Tonsillektomie unsicher.

Fall 25. Ausserordentlich schwerer Fall, bei dem das Sternalpunktat und das Blutbild eine totale myeloische Aplasie erkennen liessen. Die Tonsillektomie wurde 8 Tage nach der Erkrankung ausgeführt, und der Patient starb einen Tag danach. Bei der Obduktion wurde eine Pneumonie festgestellt. Der Verf. hält die Tonsillektomie bei derartigen Fällen für kontraindiziert.

*Fall 26. Schwerer Fall, bei dem die Tonsillektomie 6 Tage nach der Erkrankung stattfand. Die Blutveränderungen verschwanden schnell, stellten sich aber wieder ein, als die Patientin 3 Tage nach der Operation eine Pneumonie und später ausserdem eine zu einer Ösophagusfistel führende Mediastinitis bekam. Exitus 11 Tage nach der Tonsillektomie. Der Verf. betrachtet die Tonsillektomie allein als eine unzureichende therapeutische Massnahme. Ausserdem wäre eine die Myelopoese stimulierende Behandlung und die Anwendung von Sulfanilamidpräparaten in grösserer Menge, als sie gebraucht wurden, notwendig gewesen.*

### C. Tonsillitis acuta-Fälle mit normaler Blutreaktion.

#### 1. Frühere Untersuchungen des Sternalpunktates und des Blutbildes.

Systematische Untersuchungen des Sternalpunktates bei Fällen mit Tonsillitis acuta habe ich im Schrifttum nicht gefunden. Möglicherweise enthält das Untersuchungsmaterial über im Knochenmark verursachte Veränderungen bei akuten Infektionen (BARTA, KLIMA, NORDENSON, ROHR, YAMAMOTO) auch Fälle mit akuter Tonsillitis. Auch die Einwirkung der im akuten Stadium ausgeführten Tonsillektomie auf das Mark ist, soviel ich sehen kann, früher nicht untersucht worden. Die Blutbefunde sind in den Fällen mit Tonsillitis acuta von ähnlicher Art wie im allgemeinen bei den akuten pyogenen Infektionen. Im Anfangsstadium findet man eine neutrophile Leukozytose und eine Linksverschiebung der Neutrophilen. Im Heilungsstadium ist oft eine relative Lymphozytose und ab und zu eine Monozytose zu konstatieren (NÄGELI, SCHILLING). Untersuchungen über die Einwirkung der Tonsillektomie auf das Blutbild bei Fällen mit Tonsillitis acuta habe ich nicht gefunden.

#### 2. Eigene Untersuchungen.

##### a) Klinische Daten.

Die Einwirkung der akuten Tonsillitis und der Tonsillektomie auf das Knochenmark und das Blutbild von Personen mit normaler Hämatopoese habe ich bei 6 Fällen untersucht. Alle diese Patienten

wurden in der Oto-Laryngologischen Klinik gepflegt. Die auf sie bezüglichen klinischen Daten sind in Tabelle 18 zusammengestellt, in der nur die positiven Befunde der Krankheitsberichte vermerkt sind.

Die Anamnese sämtlicher 6 Patienten weist auf eine langwierige und unzweideutige Tonsillitis hin. Fünf von ihnen hatten früher ein oder mehrere Male einen Peritonsillarabszess gehabt. Es handelte sich also bei allen um eine auf der Basis rezidivierender akuter Entzündungen entstandene chronische Tonsillenerkrankung, die sich bei der Aufnahme der Patienten wieder bis zum akuten Stadium entwickelt hatte. Im Krankenhaus wurde bei 4 von ihnen (29, 30, 31 und 32) ein Peritonsillarabszess festgestellt, und bei einem (28) zeigten sich bei der Operation deutliche peritonsilläre entzündliche Veränderungen. Nach der Tonsillektomie verlief die Heilung bei allen Fällen rasch. Das Fieber verschwand in 2—3 Tagen. Ein solches schnelles Abklingen der Infektionssymptome bei einer im akuten Stadium ausgeführten Tonsillennukleation entspricht der Regel (MEURMAN, SALINGRE, VIRTANEN). Als Komplikation der Angina trat bei einem Patienten (28) eine Nephritis auf, die jedoch schnell heilte, so dass die Harnveränderungen 10 Tage später ganz verschwunden waren. Bei einer Patientin (29) erschienen ein paar Wochen nach der Operation Arthritissymptome. Diese heilten im Lauf von etwa 2 Monaten.

#### b) Knochenmarks- und Blutbefunde.

Vor der Tonsillektomie waren die Sternalpunktate (Tabelle 19) durchgängig bemerkenswert zellenreich. Das Verhältnis der Erythroblasten zu den Leukozyten zeigt, dass der ungewöhnlich grosse Zellgehalt auf einer Vermehrung der myeloischen Zellen beruht. Dieses Verhältnis betrug bei meinen Patienten durchschnittlich 1:13, entsprechend dem Index 1:6 bei meinen Normalfällen. In den gegenseitigen Beziehungen der verschiedenen Zellgruppen waren den Normalwerten gegenüber Veränderungen zu konstatieren, deren Umfang bei verschiedenen Fällen verschieden war. Die Mengen der Eosinophilen (sowohl der Prototypen als der reifen) waren im Mittel geringer als gewöhnlich. Hinsichtlich der Myeloblasten war keine deutliche Abweichung zu er-

Nr.	Alter	Ge- schlecht	Beruf	A n a m n e s e	Allgemeinzustand
27	26	♂	Arbeiter	(Ohrenklin. Journ.-Nr. 153/39.) 5-J. Anginen 2—3 mal jährlich. 1936 nach Angina eine 4 W. dauernde Nierenentzündung. Keine Peritonsillarabszesse. Am 21. 8. 1939 erkrankte Pat. mit Fieber und Rachenschmerzen, welche Symptome fortbestanden.	23. 8. 1939. Allgemeinzustand mitgenommen. Temp. 39.4°. Innere Organe: o. B.
28	33	♂	Lagerarbeiter	(Ohrenklin. Journ.-Nr. 162/39.) 1922 Osteomyelitis am r. Unterschenkel. Damals mehrere Male operiert. In den letzten Jahren mindestens 9 mal Anginen. Vor 2 J. ein Peritonsillarabszess. 2. 9. 1939. Fieber und Rachenschmerzen. 3. 9. Rückenschmerzen und Trübung des Harnes.	4. 9. 1939. Allgemeinzustand gut. Temp. 38.5°. Am Unterschenkel Operationsnarben. RR 150/90. Innere Organe: o. B. Harn: Eiw. +. Sedim.: Ziemlich reichlich rote und weisse Blutkörperchen. Keine Zylinder.
29	27	♀	Arbeiterfrau	(Ohrenklin. Journ.-Nr. 234/40.) Seit 1933 oft Anginen. Einmal ein Peritonsillarabszess. Am 21. 11. 1940 erkrankte Pat. mit Fieber und Rachenschmerzen. Schlucken und Sprechen erschwert.	26. 11. 1940. Allgemeinzustand mitgenommen, blass. Temp. 38.2°. Innere Organe: o. B.
30	26	♀	Hausangestellte	(Ohrenklin. Journ.-Nr. 190/41.) Während 3—4 J. mehrere Anginen und in Verbindung damit 2—3 mal ein Peritonsillarabszess. Am 1. 3. 1941 erkrankte Pat. wieder mit Rachenschmerzen, Fieber und zunehmenden Schlingbeschwerden.	4. 3. 1941. Allgemeinzustand mitgenommen. Temp. 38.9°. Innere Organe: o. B.
31	18	♂	Feiler	(Ohrenklin. Journ.-Nr. 261/41.) Hat früher mehrere Anginen gehabt. 2 mal Peritonsillarabszess. Am 3. 3. 1941 bekam er Fieber und Rachenschmerzen, die fortbestanden. Vor 2 Tagen begann das Öffnen des Mundes und das Sprechen schwer zu werden.	9. 3. 1941. Allgemeinzustand gut. Temp. 38.0°. Innere Organe: o. B.
32	43	♂	Arbeiter	(Ohrenklin. Journ.-Nr. 261/41.) Vor 15 J. wurde im r. Ohr eine Radikaloperation ausgeführt. Etwa 15 J. jährlich mehrere Male Anginen. Mindestens 2 mal ein Peritonsillarabszess. Eine W. lang bestanden Schmerzen im Schlunde und Fiebersymptome. In den letzten Tagen zunehmende Schlingbeschwerden.	1. 4. 1941. Allgemeinzustand gut. Temp. 37.6°. Innere Organe: o. B.

Tonsillenstatus	Zeitpunkt der Tonsillektomie und Operationsbefunde	Decursus
Daumenspitzen-gross, stark gerötet, auf der Oberfläche einige weisse Flecke.	23. 8. 1939. Die Tonsillen lassen sich leicht ablösen. Eine peritons. Entzündung ist nicht festzustellen. R. Tonsille walnussgross, l. kleiner.	Nach der Operation an 2 Tagen fieberfrei. 2. 9.—39. Befinden völlig gut, Operationswunden gut geheilt.
Schlund, Uvula und Gaumenbögen gerötet. Tonsillen pflaumengross, gerötet, auf beiden weisse Tüpfel. Leichte peritonsilläre Schwellung.	5. 9. 1939. Beiderseits starke entzündliche Veränderungen um die Tonsillenkapsel und peritonsillär. Auf der Oberfläche der Kapsel eitrige Beläge, kein eigentlicher Abszess.	Nach der Tonsillektomie verschwand das Fieber in 1 ½ Tagen. 14. 9. 1939. Befinden gut. Harn: o. B.
Öffnen des Mundes erschwert. Rachen und Tonsillen gerötet. Beiderseits starkes peritons. Ödem. In beiden Mandibularwinkeln ein paar kleine empfindliche Drüsen.	26. 11. 1940. Bei der Ablösung der r. Tonsille öffnet sich eine Eiterhöhle, die lateralwärts vom unteren Pole liegt. Die ganze Tonsillenkapsel verdickt. Bei der Ablösung der l. Tonsille wird ein kleiner Abszess oben lateral hinter der Kapsel festgestellt. Auch die Kapsel der l. Tonsille stark verdickt.	Nach der Tonsillektomie sank das Fieber in 2 Tagen, und der Allgemeinzustand besserte sich schnell. Ein paar Wochen nach der Operation bekam Pat. zunehmende, vom einen Gelenk zum anderen wechselnde Schmerzen. In den beiden Handgelenken war Tastempfindlichkeit zu konstatieren. Pat. bekam Salicyl und R-Be-handlung der Handwurzeln. Die Gelenksbeschwerden verschwanden allmählich im Lauf eines Monats. Nachuntersuchung am 6. 3. 1941: Symptomlos.
Öffnen des Mundes erschwert. Weicher Gaumen, Uvula und Gaumenbögen gerötet und geschwollen. R. Tonsille daumenspitzen-gross, belegt. L. bis in die Mittellinie verschoben. Ihre Oberfläche belegt. Vorderteil des l. Gaumenbogens vorgebuchtet. In den Kieferwinkeln daumenspitzen-grosse, ziemlich empfindliche Drüsenpakete.	5. 3. 1941. Die Tonsillen daumenspitzen-gross, gedunsen. Lateralwärts von der l. Tonsille ein grosser peritonsillärer Abszess. Hinter der r. eine kleine Eiterhöhle, worin 1—2 cm <sup>3</sup> Eiter. Die Tonsillen lösen sich mässig leicht ab.	Das Fieber verschwand innerhalb 2 Tagen nach der Operation. Heilung der Wunden normal.
Weicher Gaumen, Gaumenbögen und Hinterwand des Schlundes gerötet und geschwollen. Starker Foetor ex ore. Tonsillen gross, rot, teilweise mit grauen Belägen. Beide Tonsillen und vordere Gaumenbögen stark nach der Mittellinie vorgewölbt, so dass sie sich hier beinahe berühren. In den Kinnladen starker Trismus.	10. 3. 1941. Beide Tonsillen daumenspitzen-gross und mit Eiterbelägen. Bei der Ablösung der l. öffnet sich ein umfangreicher peritons. Abszess. R. Tonsille etwas adhären, lässt sich jedoch stumpf ablösen. Ausserhalb ihrer Kapsel, am hinteren Gaumenbogen, ein 1—2 cm <sup>3</sup> Eiter enthaltender Abszess.	Nach der Tonsillektomie sank das Fieber in 2 Tagen. Heilung der Operationswunden normal.
Weicher Gaumen, Gaumenbögen und Hinterwand des Schlundes gerötet. Links eine ziemlich starke peritonsilläre Anschwellung. Tonsillen daumenspitzen-gross, grubig, gerötet.	1. 4. 1941. Hinter dem oberen Pol der l. Tonsille öffnet sich eine fingerspitzen-grosse Eiterhöhle. Beide Tonsillenkapseln ziemlich stark narbig. Gewebe der Tonsillen gewöhnlich.	Nach der Operation einen Tag afebril. Heilung der Wunden normal.

**Tabelle 19.**  
Die Sternalpunkte der gewöhnlichen Angina-Fälle.

Die Sternalpunkte der Gewinnschicht

Nr.	Datum der Tonsillektomie	Datum	%										Zahl auf 400 L								
			Bas. Myeloz. & Leukoz.	Eos. Myeloz.	Eos. Leukoz.	Myelobl.	Neutr. Promyeloz.	Neutr. Myeloz.	Neutr. Metamyeloz.	Neutr. stabk. Leukoz.	Neutr. segm. Leukoz.	Lymphoz.	Monoz.	Retikulum- & Plasmaz.	Megakar.	Normobl.	Makrobl.	Proerythrobl.	Rote Mitosen	Weisse Mitosen	
27	23/8—39	23/8—39	0	0.50	1.25	0.50	6.25	8.0	8.50	21.50	38.50	14.75	0.25	13	0	27	3	0	0	0	
		24/8	0	0	0.25	2.0	9.0	5.75	26.75	36.75	19.25	0	9	0	3	1	0	0	0		
		1/9	0.25	2.0	0.75	5.25	15.0	19.75	20.50	18.50	15.25	1.75	22	0	36	8	2	1	0		
28	5/9—39	5/9	0.25	2.0	1.0	0.50	9.25	12.0	11.25	15.25	25.25	19.75	3.50	18	0	32	3	0	0	0	
		7/9	0	1.0	0.25	1.75	13.0	11.25	20.0	36.75	14.25	1.25	10	0	27	3	0	0	0		
		13/9	0.25	2.0	0.25	3.50	10.50	15.0	17.75	25.50	23.25	1.25	8	0	41	1	0	0	0		
29	26/11—40	26/11—10	0	1.0	0.50	0	7.75	8.75	13.0	14.25	45.25	9.50	0	8	0	15	1	0	0	0	
		29/11	0	0.50	0.75	1.25	5.75	23.25	28.0	8.50	10.50	20.0	1.50	9	0	19	5	0	0	0	
		5/12	0	2.75	0	0.75	5.0	19.75	31.0	13.25	19.75	7.25	0.50	4	0	14	4	0	1	0	
30	5/3—41	2/1—41	0	1.0	1.25	1.25	3.0	12.50	15.75	6.75	22.0	34.75	1.75	10	0	83	9	0	2	0	
		6/3	0.25	1.75	0.50	4.50	18.0	19.0	11.75	19.75	22.0	1.50	12	0	52	6	0	0	1		
		5/3	0.50	3.0	0.75	8.75	15.25	14.75	12.50	21.25	20.25	2.25	27	0	29	1	0	1	0		
31	10/3—41	7/3	0	3.0	0.25	0.50	9.50	25.50	26.0	14.75	8.0	12.50	0	23	0	14	3	0	0	0	
		10/3	0.25	2.50	0.25	14.75	22.75	13.25	12.0	18.0	14.25	1.25	10	1	27	3	0	1	1		
		13/3	0.25	0.50	0	7.25	22.25	26.75	14.25	7.25	19.50	1.25	16	0	23	0	0	0	0		
32	1/4—11	1/4	0.25	0.75	0	0.75	10.50	22.75	16.75	10.0	11.50	26.0	0.75	18	2	41	9	0	0	0	
		3/4	0	2.25	0.25	1.0	6.75	18.0	17.75	9.75	9.25	31.50	3.50	11	0	31	6	0	2	0	

kennen. Die Zahl der Promyeloblasten war erhöht. In den Fällen 31 und 32 lag sie ausserhalb der normalen Variationsgrenze, und bei den anderen 4 Patienten belief sie sich im Mittel auf 8 %, entsprechend 5 % des normalen Mittelwertes. Die Mengen der Myelozyten bis zu den segmentkernigen Leukozyten schwankten, bald waren sie vermehrt, bald vermindert oder zeigten normale Höhe. Lymphozyten waren bei meinen Fällen durchschnittlich 17 % vorhanden. Ihre Menge zeigt im Vergleich zu der normalen (14 %) eine Neigung zuzunehmen. Retikulum- und Plasmazellen wurden zahlreicher als gewöhnlich und zwar durchschnittlich 14 je 400 Leukozyten angetroffen. Wie das am Anfang erwähnte Verhältnis zu erkennen gibt, war die Menge der erythroischen Zellen zugunsten der myeloischen vermindert.

Von den oben angeführten Veränderungen sind die myeloische Hyperplasie, die Eosinopenie, die Linksverschiebung der Neutrophilen und die Zunahme der Retikulumzellen Reaktionen, wie sie von den akuten Infektionen gewöhnlich im Mark hervorgerufen werden und die aus früheren Untersuchungen bekannt sind. Die Stärke der Reaktion kann im Zusammenhang mit der Angina bedeutende Masse annehmen. KLIMA sowie NORDENSON betrachten die infektiöse Reaktion als stark, wenn die Gesamtmenge der Promyelozyten und Myelozyten im Mark 30 % übersteigt. In meinem Fall 31 fanden sich von diesen Zellen zusammen 38 % und in Fall 32 33 %. Das Knochenmarksbild entsprach bei diesen Patienten YAMAMOTO's «reifem Promyelozytenmark» sowie BARTA's «starker — sehr starker Reaktion». Die Punktrate der Fälle 27 und 29 zeigten eine Vermehrung der reifen Neutrophilen (Segment- und Stabkernigen), wobei es sich nach BARTA um eine «mässige Reaktion» handelt. In den Fällen 28 und 30 standen die Werte der myeloischen Zellgruppen den normalen Mittelwerten näher als bei den anderen Patienten; doch war die Zahl der Promyelozyten auch bei diesen hoch. ROHN erwähnt, er habe bei schweren chronischen Infektionen Knochenmarksbilder angetroffen, für die einerseits eine Zunahme der Promyelozyten und andererseits eine solche fertiger Zellen kennzeichnend wäre. Wenigstens in Fällen 27 und 29 liegt ein ziemlich ähnliches Knochenmarksbild vor. Ich möchte dies betonen, da es sich in meinen Fällen nicht um eine akute Infektion bei früher gesunden Patienten, sondern um das akute Stadium einer chronischen Infektion handelte. —



Tabelle 20.  
Die Blutbilder der gewöhnlichen Angina-Fälle.

Nr.	Datum der Tonsil- lektomie	Datum	Hb	Erythroz.	Index	Leukoz.	Neutroph.	Meta- myeloz.	Stab- kernige	Segment- kernige	Basoph.	Eosinoph.	Monoz.	Lymphoz.	Throm- boz.	SR
27	23/8—39	23/8—39	84	4700	0.88	15900	73.5	0	3.5	70.0	1.0	0.5	3.5	21.5		33
		24/8	82	4500	0.91	15700	78.0	0	6.0	72.0	1.0	0	4.0	17.0		65
		26/8	80	4740	0.85	10450	49.5	0	3.0	46.5	0	0.5	19	31.0		50
		2/9	80	4610	0.87	9600	61.0	0	9.0	52.0	0	1.5	4.0	33.5		27
28	5/9—39	4/9	78			12000	69.5	0	4.5	65.0	0	1.5	9.5	19.5		
		6/9	92	5260	0.89	12300	69.0	1.0	14.5	53.5	0.5	2.5	5.0	23.0		
		8/9	78	5180	0.76	9850	65.5	0.5	8.0	57.0	0.5	3.5	9.0	21.5		21
		13/9	87	5040	0.87	6100	63.0	0	2.5	60.5	0.5	1.5	9.5	25.5		
29	26/11—40	26/11—40	78/84	4530	0.92	17600	83.2	0	7.0	76.2	0	0	1.4	15.4	271000	49
		29/11	68/73	4400	0.83	9800	64.6	0	9.6	55.0	0.2	1.8	7.4	26.0	224400	57
		5/12	75/81	4400	0.92	16700	72.0	0	11.0	61.0	0	1.8	3.0	23.2	280000	33
		2/1—41	76/82	4350	0.94	12500	48.4	0	2.0	46.4	0.2	3.6	6.4	41.4	426000	9
		6/3	75/81	4370	0.94	9500	61.8	0	2.8	59.0	0	1.4	4.6	32.0	152900	7
30	5/3—41	5/3	75	4880	0.78	22800	86.0	0	4.0	82.0	0	0	4.5	9.5		36
		6/3	75	4740	0.79	12600	76.5	0	4.0	72.5	0.5	1.5	3.5	18.0		51
		7/3	74	4370	0.80	7300	64.0	0	2.0	62.0	0	5.5	6.5	24.0		39
		8/3	76	4430	0.85	7200	56.0	0	1.0	55.0	0.5	3.5	6.5	33.5		17
31	10/3—41	10/3	83	4980	0.84	21700	74.0	0	3.5	70.5	0	0.5	7.0	18.5		31
		11/3	82	4650	0.89	12200	74.5	0	2.5	72.0	0.5	1.5	7.0	16.5		41
		12/3	82	4990	0.82	8600	57.0	0	3.0	54.0	2.5	2.0	4.0	34.5		27
		13/3	82	5100	0.80	10800	56.0	0	2.5	53.5	0	2.0	6.0	36.0		19
32	1/4—41	1/4	89	4690	0.90	14700	77.0	0	2.0	75.0	0	0	4.5	18.5		25
		3/4	82/89	5230	0.84	11200	75.8	0.2	13.8	61.8	0.6	0.6	6.0	16.8		61

Das Knochenmarksbild in Fall 28, in dem als Komplikation eine Nephritis vorlag, wich nicht besonders von den anderen ab.

Die Neigung zur Vermehrung der Lymphozyten ist ein Befund, der nicht zu dem von akuten Infektionen hervorgerufenen Reaktionsbild gehört. KLIMA und ROHR haben bei anderen akuten Infektionen im Gegenteil bemerkenswert niedrige Lymphozytenwerte festgestellt. Das erklärt sich aus der myeloischen Hyperplasie des Knochenmarkes. Ebenso wie die Menge der Erythroblasten im Punktat auf Kosten der myeloischen Zellen abnimmt (was sich in der Veränderung des Erythroblasten-Leukozytenindex äussert), werden auch die Werte der Lymphozyten kleiner. Dass in den Lymphozytenwerten meiner eigenen Fälle diese gewöhnliche Abnahme trotz der bedeutenden myeloischen Hyperplasie nicht nachzuweisen ist, lässt erkennen, dass die Menge der Lymphozyten bei den Patienten grösser als gewöhnlich gewesen ist. Dies ist eine Feststellung, die früher nicht im Schrifttum gemacht worden ist. In diesem Punkt entsprechen meine Ergebnisse den Befunden bei Fällen mit Tonsillitis chronica, bei denen auch eine Vermehrung der Lymphozyten auftrat, und bestätigen die herrschende Auffassung, dass die akute und die chronische Tonsillitis oft verschiedene Phasen ein und derselben Krankheit sind.

Das weisse Blutbild (Tabelle 20) zeigte vor der Tonsillektomie, wie zu erwarten war, eine neutrophile Leukozytose. Die Gesamtmenge der weissen Blutkörperchen schwankte bei meinen Patienten zwischen 12000 und 22800. Zwischen dem Betrag der Leukozytose und der Stärke der Knochenmarksreaktion ist kein Parallelismus festzustellen. Trotz der Leukozytose war die Linksverschiebung der Neutrophilen relativ gering. Metamyelozyten kamen bei keinem Patienten vor, und die Zahl der Stabkernigen war nur in einem Fall (29) erhöht. Durchschnittlich waren stabkernige 5 % vorhanden. Ausserdem zeigte das weisse Blutbild in 3 Fällen eine Eosinopenie.

Das rote Blutbild stand bei allen Patienten auf normaler Höhe.

Die Sternalpunktion wurde 1—3 Tage nach der Tonsillektomie ausgeführt. Die myeloische Hyperplasie war da noch stärker als vor der Operation. (Der Erythroblasten-Leukozytenindex war 1:18.) Unter den Veränderungen, die früher in den gegenseitigen



### c) Besprechung der Ergebnisse.

Die Fälle zeigen, dass die akute Tonsillitis im Knochenmark einen deutlichen Reizzustand hervorruft, dessen Stärke individuell wechselt und der ebenso grosse Masse erreichen kann wie z. B. bei Pneumoniefällen. Die Veränderungen waren ähnlicher Art, wie sie in der früheren Literatur im allgemeinen bei anderen akuten Infektionen angetroffen worden sind. Von der gewöhnlichen Reaktion abweichend, traten jedoch Zeichen auf, die auf eine Erhöhung der Lymphozytenzahl im Mark hinwiesen. Ferner ergab sich als neuer Befund, dass die Tonsillektomie eine Verstärkung der schon vorher bestehenden Reaktion bewirkte. Von dem durch die Operation ausgelösten zusätzlichen Reiz zeugte auch die gleichzeitige Zunahme der Linksverschiebung der Neutrophilen im peripheren Blut. Nach der Tonsillektomie verschwanden die infektiösen Blutveränderungen etwa im Lauf einer Woche. Im Knochenmark schien die Reaktion etwas länger zu dauern.

Aus den Tonsillitis acuta-Patienten wollte ich ein Kontrollmaterial zusammenbringen, mit dem ich die oben besprochenen Granulozytopenie-Fälle vergleichen konnte. Trotz einer klinisch ähnlichen Tonsilleninfektion waren die hämatologischen Veränderungen in beiden Gruppen einander entgegengesetzt. Im Mark zeigte sich bei den Granulozytopenie-Patienten eine Behinderung der Leukopoese und in den gewöhnlichen Anginafällen umgekehrt eine beschleunigte Funktion. Die Veränderungen des Blutbildes bei der Granulozytopenie-Angina bildeten gewissermassen ein Spiegelbild der normalen Reaktion. Der Effekt der Tonsillektomie war in den Fällen beider Gruppen der gleiche. Die klinischen Symptome verschwanden nach der Operation rasch, und die einander entgegengesetzten hämatologischen Veränderungen erloschen ungefähr nach gleich langer Zeit. Mein Material an gewöhnlichen akuten Tonsillitiden sowie an Granulozytopenie-Anginen ist jedoch zu klein, als dass ich auf Grund desselben endgültig Stellung zu der Frage nehmen könnte, wie oft sich mit der Tonsillektomie eine derartige günstige klinische und hämatologische Wirkung verbindet.

Im Lichte des normalen Reaktionstypus ist es möglich, die Wirkungsart der Tonsillektomie auf die Granulozytopenie-Anginen genauer zu beurteilen. (In diesem Zusammenhang habe ich Fall 25

unberücksichtigt gelassen.) Es können zwei Alternativen in Betracht kommen. Erstens kann die Tonsillektomie auf die Myelopoese als unspezifischer Reiz wirken. Es hat sich nämlich herausgestellt (JUNG, STAHL), dass die chirurgischen Massnahmen (auch die aseptischen) im allgemeinen im Blutbild eine neutrophile Leukozytose und mithin wahrscheinlich auch im Knochenmark eine Reaktion verursachen. Wie erwähnt, trat im Mark meiner Anginapatienten ein derartiger durch die Tonsillektomie ausgelöster Reiz auf. Ein solcher war wahrscheinlich auch als zur Heilung der Krise beitragender Faktor bei meinen Granulozytopenie-Fällen vorhanden, obwohl er infolge des pathologischen Charakters der Markreaktion nicht auf dieselbe Weise wie in den Anginafällen zum Vorschein kam. Die andere in Betracht kommende Alternative ist, dass die mittels der Tonsillektomie erreichte Beseitigung des Infektionsherdes die spezifische Ursache darstellt, die zur Unterbrechung der Krise führt, ebenso wie sie bei den Anginapatienten das schnelle Verschwinden der Leukozytosenreaktion nach dem vorausgehenden zusätzlichen Reiz bewirkt. Das Abklingen des Fiebers und die rasche Besserung des Allgemeinzustands sprechen meines Erachtens dafür, dass hauptsächlich diese Alternative wirksam war.

#### d) Zusammenfassung.

*Die Einwirkung der akuten Tonsillitis und der während derselben ausgeführten Tonsillektomie auf die Hämatopoese wurde in 6 Fällen untersucht. Vor der Tonsillektomie zeigte das Knochenmark eine myeloische Hyperplasie, eine Linksverschiebung der myeloischen Zellen, eine Eosinopenie und eine Vermehrung der Retikulumzellen. Die Zahl der Lymphozyten im Mark war grösser, als es bei akuten Infektionen im allgemeinen der Fall ist. In den Blutbildern trat eine neutrophile Leukozytose auf, an die sich eine leichte Linksverschiebung angeschlossen hatte. In den ersten Tagen nach der Tonsillektomie war die infektiöse Reaktion des Knochenmarkes noch stärker als vorher und die Linksverschiebung im peripheren Blut gesteigert. Die Veränderungen des Blutbildes verschwanden nach der Tonsillektomie schnell, obwohl nicht so schnell wie das Fieber. Die Veränderungen im Sternalpunktat glichen sich langsamer aus, denn sie bestanden teilweise noch 10 Tage nach der Tonsillektomie.*

## D. Die Fälle mit Tonsillitis chronica.

### 1. Frühere hämatologische Untersuchungen.

Knochenmarksuntersuchungen bei chronischer Tonsillitis habe ich in der früheren Literatur nur einmal gefunden. KLIMA erwähnt nämlich einen Fall, in dem das Mark bei normalem Differentialbild der Leukozyten sehr zellenreich war.

Dagegen sind viele Untersuchungen über das Blutbild in dem umfangreichen Schrifttum anzutreffen, das sich mit der Fokalinfection beschäftigt. Durch systematische Untersuchungen hat man die Fragen aufzuhellen versucht, ob lokale Entzündungs-herde Reaktionen im Blutbild hervorrufen und ob die Bestimmung desselben bei der Diagnose der Fokalerkrankungen von Nutzen ist. Die meisten dieser Arbeiten sind in Fällen ausgeführt worden, bei denen nur Zahnherde vorhanden waren, und nur wenige behandeln ausschliesslich die Veränderungen, die durch chronische Tonsillitis im Blutbild verursacht werden. Eine allgemeine Vorstellung von den zuletzt erwähnten Untersuchungen ebenso wie von denen, die die durch einen Fokalherd im Blutbild bewirkten Veränderungen im allgemeinen betreffen, gibt Tabelle 36 auf Seite 136.

Aus den angeführten Untersuchungen geht hervor, dass das Blutbild bei chronischer Tonsillitis normal sein kann. Oft kommen darin jedoch pathologische Veränderungen vor, obwohl die Abweichungen vom Normalen im allgemeinen nicht sehr erheblich sind. Über das rote Blutbild finden sich nur wenig Angaben. Einige Autoren haben keine Veränderungen darin konstatiert, andere dagegen eine Neigung zu hypochromer Anämie. Lymphozytose und Linksverschiebung der Neutrophilen sind die Veränderungen des weissen Blutbildes, die am häufigsten festgestellt wurden. Die Ergebnisse der verschiedenen Forscher stimmen nicht überein, sondern divergieren vielmehr in manchen Punkten. Das zeigt sich auch in den zahlreichen Untersuchungen, die den Einfluss der Zahnherde auf das Blutbild berühren (BRYANT und POLEWITSKY, McCONNEL, GOLOWANOWA und Mitarbeiter, HADEN, HARVEY und Mitarbeiter, HECKER, HUME, LINKNER, LOGAN, NIEHAUS, POTTER und Mitarbeiter, PRICE, RYGG, TOREN). Zu der Verschiedenheit der Resultate hat augenscheinlich teilweise die ungleiche Art der Untersuchungsmaterialien beigetragen. Aus

den Veröffentlichungen ist oft schwer zu ersehen, welches das klinische Krankheitsbild der Fälle gewesen ist. Es wird nicht immer hinreichend klar, ob die Patienten ausser den Entzündungsherden auch von diesen hervorgerufene Sekundärkrankheiten hatten. Von Bedeutung für die Art und Stärke der Veränderungen ist es auch, in welchem Stadium sich die Fokalinfection des Patienten befindet. Es ist allgemein bekannt, dass der klinische Verlauf einer Fokalinfection wellenförmig ist. Der Herd kann lange latent oder isoliert bleiben und nur zeitweise Zeichen eines aktiven Prozesses geben. Im ganzen deuten diese Untersuchungen darauf hin, dass die chronische Tonsillitis und die periapikalen Zahninfektionen oft auf das Blutbild einwirken. Das Bild, das man mit ihrer Hilfe von der Hämatopoese erhalten hat, ist jedoch unvollständig.

Die Reaktion, welche die im »kalten« Stadium ausgeführte Tonsillektomie im Blutbild ausgelöst hat, ist von ANDROLI und von CALABRESI studiert worden. Nach der Operation findet man Leukozytose, Linksverschiebung und relative Lymphopenie, Veränderungen, die nach dem erstgenannten Autor schon nach einem Tag und nach dem letztgenannten nach 4—5 Tagen verschwinden.

## 2. Eigene Untersuchungen.

### a) Klinische Daten.

#### Allgemeines.

Mein Material an chronischen Tonsillitiden habe ich in der Medizinischen Poliklinik der Universität gesammelt. Die Tonsillektomie wurde in der Oto-Laryng. Klinik ausgeführt, von wo die Patienten unmittelbar nach der Operation in die klinische Abteilung der Poliklinik zurückgeschickt wurden. (Die beiden Kliniken liegen nahe beieinander.)

Von den Patienten waren 30 Frauen und 15 Männer. Das mittlere Alter betrug 25.7 Jahre.

Wie früher erwähnt, wurde bei allen Patienten eine genaue klinische Untersuchung vorgenommen. In der Anamnese wurde besonders auf das Vorkommen von Infektionen geachtet und

of such reactions. This finding is in accord with that of Hartung (1943) and Secher (1946). Especially in winter and in spring in our country it is necessary to supplement the diet by vitamin C tablets or compounds. Infections, such as rheumatoid arthritis, cause a fall in the ascorbic acid content of the blood, Rinehart et al. (1936), Secher (1946). Low values in ascorbic acid are to be found especially in winter and spring in the northern countries (Harrestrup-Andersen & Normann, 1945). Our experience goes in the same direction as that of Secher: since the introduction of vitamin C therapy 10 years ago we have had no case of thrombocytopenia (under 100,000/c.m.).

The large retention of gold during its administration is an important thing that must be borne in mind. The mean excretion of gold in the urine — about 80 per cent of the total excretion (Freyberg et al., 1942) in the case of these gold salts — has in this series shown values between 1.40 and 0.40 mg per diem or between 10 and 3 mg per week. At the same time between 120 and 30 mg of Aurum have been given per week. During the course of gold therapy with such doses, about 80—90 per cent of the injected gold salt apparently remains in the body, and we have found gold in the plasma six months after the last injection of gold in this series (cf. Freyberg et al., 1941, Comroe, 1944).

The treatment of the reactions following chrysotherapy has up to the present been purely symptomatic. In the case of skin reactions we have given 2 g of calcium gluconate 3 times daily per os and 10 ml of 10 per cent solution of calcium gluconate intravenously daily. Sodium thiosulphate is also given intravenously, 10 ml daily in 10 per cent solution. For the special skin-treatment Boeck's liniment and other ointments. Atropine for the pruritus.

This treatment has not been of so good effect. The reactions, especially the complications from the skin, kidneys and blood, have often been of considerable duration, severity and capriciousness. For that reason the new treatment of such reactions with BAL (2,3-dimercaptopropanol) has been greeted with delight.

Recently we have had an opportunity of treating two cases of gold-dermatitis with BAL.

The first case was a man of 37 years with a relatively fresh rheumatoid arthritis (S. R. 40/1 h., AST neg., Agglutination against haemolytic streptoc. pos.), who was given in all 0.35 g of Myoral (aurothioglucanate). Three days after the last i. m. in-



Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
33	32	♀	Bauersfrau	(Nr. 72/39.) <sup>1</sup> Während eines Jahres sind übelriechende »Graupen« in den Mund aufgestiegen. Oft Schmerzen im Schlund. Andauernd müde. Ein paar Mon. lang wechselnde Schmerzen besonders im Rücken und in den Schultern.	18. 4. 1939. O. B.	Daumenspitzen-gross, grubig, zahlreiche Pfröpfe.
34	26	♀	Hausangestellte	(Nr. 79/39.) Mit 16 J. ½ J. Gelenkrheumatismus. Damals wurde ein Klappenfehler im Herzen festgestellt. Während der 2 letzten J. Atemnot bei Anstrengungen. 2 Mon. lang wieder wechselnde Schmerzen in den Gelenken, hauptsächlich in den Knien und Fusswurzeln. Im Rachen ein paar J. lang oft Empfindlichkeit. Pat. hat keine fieberhaften Anginen gehabt.	26. 4. 1939. Allgemeinzustand mässig gut. An den Fusswurzeln und Knien Tastempfindlichkeit. Über dem Herzen ein rauhes syst. Geräusch. Über der Spitze ein diast. Geräusch. Röntgen: L. Vorhofbogen prominierend. Retrokardialraum in seinem oberen Teil verschmälert. Ekg. o. B.	Fingerspitzen-gross, gerötet. In beiden Pfröpfe.
35	11	♂	Schüler	(Nr. 87/39.) Während eines Jahres Rachen hin und wieder empfindlich und schmerzhaft. Neigung zu Schnupfen, Husten und Temperaturerhöhungen. Andauernd müde. Vor 3 Mon. Masern ohne Komplikationen.	10. 5. 1939. O. B.	Gross, grubig. In beiden Sekret.
36	17	♂	Arbeiter	(Nr. 94/39.) Als Kind Milchgrind. Mehrere J. Neigung zu Schnupfen. Vor 5 Mon. Angina. Bekam bald danach abends beim Schlafengehen asthmatische Anfälle, die etwa 1 Std. dauerten. Vor 2 Mon. wieder Angina, wonach die asthmatischen Anfälle einige Zeit schwerer.	20. 5. 1939. Allgemeinzustand gut. In den Lungen spärlich pfeifendes Rasseln.	Daumenspitzen-gross, grubig. In beiden Pfröpfe.

<sup>1</sup> Die Nummern in dieser Tabelle beziehen sich auf das Journal der klinischen Abteilung der Poliklinik.

## 21.

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillektomie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
O. B.	Müdigkeit. Gelenkbeschwerden.	19. 4. 1939		
Einige kariöse Zähne. Röntgen: Keine periapikalen Veränderungen.	Stenosis ostii atrioventr. sin. et insuff. valv. mitralis. Polyarthrit. subacuta.	29. 4. 1939	Daumenspitzen-gross, narbig, besonders die linke.	6. 1. 1941. Teilt brieflich mit, dass sie nicht zur Nachuntersuchung kommen kann. Hat sich nach der Tonsillektomie sonst wohl gefühlt, aber fortgesetzt Herzbeschwerden.
O. B.	Müdigkeit.	13. 5. 1939		28. 8. 1939. Symptomlos. Seit der Operation keine Infektionen.
O. B.	Asthma bronchiale.	24. 5. 1939	Narbig, sehr fest mit der Umgebung verwachsen, die l. daumenspitzen-gross, die r. etwas grösser. Nach der Operation an 2 Tagen subfebrile Temperaturerhöhung.	9. 12. 1940. Nach der Tonsillektomie keine asthmatischen Anfälle.

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
37	43	♂	Landwirt	(Nr. 1751/39. Krankenhaus Mehiläinen.) Hatte in jungen Jahren mehrere Anginen, vor 14 J. nach Angina eine Nierenentzündung, die bei Anstaltspflege schnell heilte. Während des letzten ½ J. oft Empfindlichkeit im Rachen und wechselnde Schmerzen in den Gelenken.	9. 6. 1939. O. B. Ekg. o. B. In den Fäzes Bothriocephalus-Eier.	Daumenspitzen-gross, gerötet, in beiden flüssiges Sekret.
38	30	♀	Bauersfrau	(Nr. 1749/39. Krankenhaus Mehiläinen.) Hatte während 12 J. mehrere Anginen. Vor 3 Mon. Morbilli. Danach Neigung zu Schnupfen, oft leichte Rachenschmerzen und zugleich unbedeutende Temperaturerhöhung. Andauernd ausserordentlich müde.	9. 6. 1939. o. B. In den Fäzes Bothriocephalus-Eier.	Fingerspitzen-gross, gerötet-grubig. Besonders in der r Sekret.
39	39	♀	Bauersfrau	(Nr. 118/39.) Seit dem 18. Lebensjahr Anginen fast regelmässig 2 mal im J. Zweimal ein Peritonsillarabszess. Zuletzt Angina vor 2 Mon. Danach fortgesetzt vom einen Gelenk zum anderen wechselnde Schmerzen.	29. 6. 1939. Allgemeinzustand gut. Tastempfindlichkeit an den Fusswurzeln, Knien, Handwurzeln, Ellbogen- und Schultergelenken.	Daumenspitzen-gross, grubig Zahlreiche Pfropfe und flüssige Sekret.
40	20	♀	Hausangestellte	(Nr. 124/39.) Vor 4 J. Angina und danach eine Nierenkrankheit und Gelenkrheumatismus, weswegen 6 W. im Krankenhaus. Danach Anginen 1—2 mal jährlich, zuletzt vor 3 Mon. Während des letzten J. besonders müde.	7. 7. 1939. O. B.	Fingerspitzen-gross, grubig In der l. Pfropfe In beiden Kiefer winkeln ein paar kleine Drüsen.
41	33	♀	Tapezierin	(Nr. 119/39.) 3—4 J. im Rachen oft Empfindlichkeit und Schlingen zeitweilig erschwert. Eigentliche fieberhafte Anginen hat Pat. nicht gehabt. In den Gelenken manchmal kurzdauernde Schmerzen. Während der letzten Mon. Neigung zu Atemnot, Herzklopfen und Müdigkeit.	16. 8. 1939. Allgemeinzustand mässig gut. Probefrühst.: Achylie.	Daumenspitzen-gross, gerötet, in beiden Pfropfe.

21 (Forts.).

Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillektomie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
3 kariöse Zähne.	Gelenkbeschwerden. Helminthiasis (Bothriocephalus).	10. 6. 1939		
Einige Zähne fehlen, die übrigen gesund.	Müdigkeit Helminthiasis (Bothriocephalus latus).	10. 6. 1939		
Mehrere kariöse Wurzeln.	Polyarthrits acuta.	30. 6. 1939		Die kariösen Wurzeln einige Tage nach der Operation extrahiert. Hat Röntgenbehandlung mehrerer Gelenke bekommen. 14. 8. 1939. Befinden gut. In den Gelenken keine Schmerzen und keine Tastempfindlichkeit.
O. B.	Müdigkeit.	8. 7. 1939	R. Tonsille sauber. In der I. Pfröpfe. Ihre Kapsel verdickt und narbig. Nach der Tonsillektomie 2 T. leichte Temperaturerhöhung.	14. 8. 1939. Symptomlos.
2 kariöse Zähne.	Anaemia hypochromica. Gelenkbeschwerden.	17. 8. 1939	Am Tage nach der Tonsillektomie leichte Temperaturerhöhung.	24. 11. 1939. Allgemeinbefinden bedeutend verbessert. Keine Gelenkbeschwerden. Hat fast regelmässig Eisen genommen.

Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
42	16	♂	Landwirts- sohn	(Nr. 146/39.) Vor 8 Mon. Masern. Vor 5 Mon. Schnupfen und Eiter in den Oberkieferhöhlen mit Genesung nach 2 Punktionen. Etwas später Angina. Vor 4 Mon. rechtsseitige Pneumonie, wobei 10 T. Fieber. Sofort danach leichte Angina. Seitdem fast andauernd Empfindlichkeit im Rachen. Pat. ist sehr müde gewesen.	21. 8. 1939. Allgemeinzustand mitgenommen. Ekg: o. B.	Gross, einander fast erreichend, grubig. In den Kieferwinkeln einige erbsengrosse, leicht schmerzhaft Drüsen.
43	17	♂	Studierender	(Nr. 145/40.) Seit dem 9. bis 10. J. mehrere Anginen. Vor 2 J. ein Peritonsillarabszess. Zuletzt Angina vor 2 Mon. Klagt über erschwertes Schlingen und andauernde Müdigkeit.	26. 6. 1940. O. B. Ekg: o. B.	Sehr gross, in der Mittellinie einander fast erreichend, grubig. In beiden reichliches Sekret.
44	33	♂	Lokomotiv- heizer	(Nr. 147/40.) Während 7 J. häufig Anginen und mehrere Peritonsillarabszesse. Zuletzt Angina und im Zusammenhang damit ein Peritonsillarabszess vor 2 Mon. Danach fortgesetzt sehr müde und wiederholt leichte Schmerzen in den Knien und Fusswurzeln.	1. 7. 1940. O. B.	Fingerspitzen-gross, gerötet, grubig.
45	20	♀	Lehrerin	(Nr. 149/40.) Während 4—5 J. mehrere Anginen, zuletzt vor 2 Mon. Danach ständige Schmerzen im Rachen und starke Müdigkeit, so dass Arbeit unmöglich.	8. 7. 1940. O. B.	Sehr gross, fast aneinanderstossend, grubig. In beiden Kieferwinkeln ein paar kleine Drüsen.
46	18	♀	Viehpflegerin	(Nr. 156/40.) Vor 9 und 3 J. schwere Angina. Das vorige J. im Rachen ab und zu leichte Schmerzen und fortgesetzt ungewöhnlich starke Müdigkeit.	18. 7. 1940. O. B. In den Stühlen Bothriocephalus-Eier.	Daumenspitzen-gross, gerötet, grubig.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillektomie	Operationsbefunde über die Tonsillen, Fleber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
O. B.	Müdigkeit.	22. 8. 1939	Hypertrophisch, besonders die l., deren Kapsel narbig ist. Am Tage nach der Tonsillektomie Temp. 37.3°.	
Zähne behandelt.	Müdigkeit.	27. 6. 1940	Ausserordentlich gross, adhärent, narbig. In ihrem Innern mehrere kleine Eiterherde.	9. 8. 1940. Befinden durchaus gut. Müdigkeit verschwunden.
2 kariöse Zähne.	Gelenkbeschwerden. Müdigkeit.	2. 7. 1940	Walnussgross, narbig, adhärent. In der l. ein bohnergrosser, dicken Eiter enthaltender Herd, in der r. ein paar kleinere Eiterherde. Nach der Operation 2 T. Temperaturerhöhung bis 37.4°.	17. 2. 1941. Befinden gut gewesen. Gelenkbeschwerden verschwunden.
2 Zähne fehlen. Einige Plomben. Keine Wurzelfüllungen.	Müdigkeit.	9. 7. 1940	Sehr gross, narbig, in beiden kleine Eiterherde. Temp. am folgenden Tage 37.3°.	15. 4. 1941. Subjektiv und objektiv symptomlos.
O. B.	Müdigkeit. Helminthiasis (Bothriocephalus).	19. 7. 1940	Walnussgross. Makroskopisch keine Eiterherde. Am Operationstag Fleber 37.8°.	21. 7. 1940. Erfolgreiche Wurmkur.

Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
47	16	♂	Arbeiter	(Nr. 158/40.) In den letzten Jahren oft Schnupfeninfektionen sowie ein paarmal fieberhafte Angina. Zuletzt Angina vor 1 Mon. Danach fortwährend Schmerzempfindung im Schlund und andauernde Müdigkeit.	29. 7. 1940. O. B.	Sehr gross, in der Mittellinie einander berührend, gerötet. In beiden etwas flüssiges Sekret.
48	22	♀	Arbeiterfrau	(Nr. 191/40.) Früher dann und wann leichte Schmerzen im Schlund. Vor 2 Mon. fieberhafte Angina. Danach andauernd hochgradige Müdigkeit, oft von einem Gelenk zum anderen wechselnde Schmerzen und leichte Temperaturerhöhung.	1. 10. 1940. Allgemeinzustand mässig gut. Temp. 37.5°. Im r. Lappen der Gl. thyroidea ein hühnereigrosses Adenom. An mehreren Gelenken leichte Tastempfindlichkeit. In den Stühlen Bothriocephalus-Eier. Ekg: o. B.	Pflaumengross, grubig. In beiden zahlreiche Pfröpfe.
49	30	♀	Bauersfrau	(Nr. 205/40.) Vor 2 J. fieberhafter Gelenkrheumatismus, der ohne bekannte Ursache begann. Wurde in 8 W. geheilt. Vor 7 Mon. Angina, die 1 Mon. später rezidierte. Sofort danach von einem Gelenk zum anderen wechselnde Schmerzen. Die Gelenksymptome bestanden weiter leicht fort. Im Schlund oft Empfindlichkeit.	21. 10. 1940. Allgemeinzustand gut. An den Fuss- und Handwurzeln Tastempfindlichkeit.	R. Tonsille pflaumengross, l. etwas kleiner, beide grubig. Besonders in der r. Pfröpfe.
50	17	♀	Schülerin	(Nr. 215/40.) Mit 7 J. Tonsillotomie. Während 2 J. mehrere fieberhafte Anginen, zuletzt vor 2 Mon., wo sie 2 W. dauerte. Oft leichte Rachenschmerzen. Menses mit dem 13. J., regelmässig, bis sie vor 1 ½ J. ganz aufhörten. In 2 J. über 5 kg abmagert. Von Zeit zu Zeit Kopfschmerz. Verdauung träge.	1. 11. 1940. Blass und ausserordentlich mager. Grundumsatz —10.	Daumenspitzen-gross, bläulich, grubig. In beiden Pfröpfe.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome außer der Ton- sillitis	Zeitpunkt der Tonsillekto- mie	Operationsergebnisse über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus, Spät-Nachuntersuchung
O. B.	Modigkeit.	30. 7. 1940	Ausserordentlich hyper- trophisch, sonst kein pathologischer Befund. Nach der Operation am 2. T. Temp. bis 37,3°.	
Mehrere kariöse Wurzeln.	Infectio rheu- matica acuta. Struma nodosa. Helminthiasis (Heterophylia- lus).	15. 10. 1940	Hypertrophisch, viele Krypten, etwas adhä- rent. Makroch. Keine Eiterherde. Nach der Operation Temp. am zwei Tagen bis 37,8°.	Vor der Tonsillektomie erfolgreiche Wurmkur und kariöse Wurzeln extrahiert, die letztere 1 W. vor der Operation 21. 1. 1941. Befinden gut. Keine Tempera- turerhöhung. Von Zeit zu Zeit Beschwerden in den Gelenken, die keine objektiven Veränderungen zeigen.
Abgestorbene Zähne: 6-- und 7-- und 6--. An den Spitzen der Wurzel von 7-- und 6-- ein pe- riapik. Granulom.	Polyarthrit. infantilis. Osti- pericarditis.	24. 10. 1940	R. Tonsille daumen- spitzengross, l. kleiner. In den oberen Teilen beider Tonsillen tiefe Gruben, worin zahl- reiche Pfropfe. In der r. einige Stellen, wo das Gewebe fester als die Umgebung. Nach der Operation am 5. T. be- deutende Temperaturerhöhung und gesteigerte Gelenk- schmerzen.	Die Zähne 7-- und 6-- 1 T. nach der Ton- sillektomie extrahiert.
1 kariöser Zahn.	Kachexia hypo- physaria. Mo- digkeit.	1. 11. 1940	Beide Tonsillen narbig, mit der Umgebung ver- wachsen, die l. mehr. L. Tonsille voll Pfropfe. Am Tage nach der Ton- sillektomie leichte Tem- peraturerhöhung.	1. 2. 1941. Allgemein- befinden nach der Ton- sillektomie bedeutend verbessert. Hat sich viel frischer gefühlt. Das Gewicht hat nicht zugenommen.



Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
51	36	♀	Fischersfrau	(219/40.) Ein paar Jahre oft wiederkehrende Empfindlichkeit im Schlunde. Während 1 J. zeitweise Schmerzen in beiden Knien. In den letzten Mon. bemerkte Pat., dass sie leichter ausser Atem kam als früher.	13. 11. 1940. Allgemeinzustand gut. Im r. Lappen der Gl. thyreoidea ein kleines Adenom. Klang der Herztöne leicht herabgesetzt. Ekg: PQ 0.22 Sek. In den Gelenken keine obj. Veränderungen.	Mandelgross. Die l. hart, voll Pfröpfe.
52	26	♀	Hausangestellte	(Nr. 228/40.) Mit 15 J. zum erstenmal Angina. Diese rezidierte danach im ganzen etwa 20 mal. Vor 2 J. Peritonissillarabszess. Vor 4 Mon. Diarrhöe mit Fieber, wonach Pat. Gelenkbeschwerden bekam. Die Knie waren am stärksten angegriffen, aus beiden wurde Flüssigkeit entfernt. Fortgesetzt leichtere Gelenkschmerzen.	20. 11. 1940. Allgemeinzustand gut. Temp. 37.3°. Bei den Kniegelenken Palpationsempfindlichkeit. Röntgenologisch in beiden Knien, besonders im r., diffuse Entkalkung. Kristensen —.	Klein, gerötet.
53	26	♀	Hausangestellte	(Nr. 243/40.) Während 4 J. mindestens 5 mal fieberhafte Angina, zuletzt vor 1 Mon., wo sie 1 W. dauerte. Danach fortgesetzt Müdigkeit und leichte, vom einen Gelenk zum anderen wechselnde Schmerzen.	11. 12. 1940. Allgemeinzustand gut. Empfindlichkeit in den Knie- und Fusswurzelgelenken. Ekg: o. B.	Fingerspitzen-gross, grubig. In beiden Kieferwinkeln ein paar leicht schmerz-hafte Drüsen.
54	16	♂	Landwirts- sohn	(Nr. 10/41.) Seit dem 7. Lebensjahr zahlreiche Anginen, zuletzt vor 10 Mon. In den letzten Mon. andauernd müde. Kann nicht arbeiten.	4. 1. 1941. Allgemeinzustand gut. Temp. 37.2°.	Kleinfingerspitzen-gross, grubig. In beiden flüssiges Sekret.
55	38	♂	Arbeiter	(Nr. 32/41.) Hatte mit 4 J. eine Nierenkrankheit. Vor ½ J. Diarrhöe, wonach andauernd Rückenschmerzen. Der Arzt stellte eine Nierenentzündung fest. Pat. hat trotz dem Rat des Arztes fortwährend gearbeitet. Vor einem Mon. Angina, wobei 3 T. Fieber. Danach der Rachen fortgesetzt empfindlich.	5. 2. 1941. Allgemeinzustand gut. Blutdr. 165/90. Folin 40 mg%. Harn: Eiw. ½°/100. Sedim.: Reichlich Erythrozyten, spärlich Leukozyten und granul. Zylinder. In den Fäzes Bothrioccephalus-Eier. Ekg: o. B.	Daumenspitzen-gross, gerötet. In der l. flüssiges Sekret. In beiden Kieferwinkeln ein paar bohnergrosse Drüsen.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillektomie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
Zahlreiche kariöse Wurzeln.	Gelenkbeschwerden. Myopathia cordis. Struma nodosa.	14. 11. 1940	In der l. Tonsille eine erbsengrosse Abszesshöhle. Tonsillen im übrigen normal.	Kariöse Zähne einige Tage nach der Tonsillektomie extrahiert. 30. 13. 1940. Befinden durchaus gut. Ekg ähnlich wie vor der Operation.
O. B.	Polyarthrititis subacuta.	21. 11. 1940	Beide Tonsillen an der Umgebung adhärent. Bei der Spaltung sind keine makroskopischen Veränderungen festzustellen.	Pat. hat nach der Tonsillektomie 3 Serien Röntgenbehandlung auf die Knie bekommen. 12. 2. 1942. Gelenke symptomlos. Befinden gut.
Mehrere kariöse Wurzeln.	Polyarthrititis acuta.	12. 12. 1940	L. Tonsille stark narbig und festgewachsen, r. frei. Nach der Operation diffuse Nachblutung.	Nach der Operation wurden die kariösen Zähne extrahiert und Röntgenbehandlung auf die Knie und Fusswurzeln gegeben. 20. 4. 1940. Allgemeinbefinden gut. In den Gelenken keine Symptome. Pat. ermüdet leicht bei der Arbeit.
1 kariöser Zahn.	Müdigkeit.	9. 1. 1941	R. Tonsille etwas adhärent, l. frei. Die Krypten sehr tief, darin Pfröpfe.	26. 3. 1941. Nach der Operation bedeutend erholt. Keine Beschwerden.
4 kariöse Wurzeln. An + 8 ein periapikales Granulom.	Nephritis acuta. Helminthiasis (Bothriocephalus). Ostitis periapicalis.	7. 2. 1941	Daumenspitzen-gross. Im Innern beider kleine Eiterherde. In den Veränderungen des Harnes keine Zunahme.	Im Laufe eines Mon. nach der Tonsillekt. wurden die schadhafte Zähne extrahiert, ausserdem eine erfolgreiche Wurmkur. 13. 3. 1942. Allgemeinzustand gut. Blutdr. 160/90. Harn: Eiw. $\frac{1}{2}$ ‰. Sedim.: einige Erythrozyten und Granul. Zylinder.

Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
56	21	♀	Ladengehilfin	(Nr. 31/41.) Mit 10 J. Tonsillotomie. In den letzten J. mehrere Anginen. Ein Peritonsillarabszess vor 2 J. und bei der letzten Angina vor 2 Mon. Etwas später Anschwellung im Gesicht, wobei eine Nephritis festgestellt wurde.	5. 2. 1941. Allgemeinzustand gut. Blutdr. 130/85. Harn: Eiw. $\frac{1}{2}$ ‰. Sedim.: spärlich Erythrozyt. und Zylinder. Folin 24 mg%. Ekg: o. B.	L. daumenspitzen-gross, r. etwas kleiner. Oberfläche grubig. In beiden Sekret. In den Kieferwinkeln ein paar erbsengrosse Drüsen.
57	31	♂	Schmied	(Nr. 37/41.) Von Jugend auf oft Anginen. Spürt bei Kälte sofort eine Empfindlichkeit im Schlund. Ein J. lang Neigung zu Herzklopfen.	12. 2. 1941. O. B. Ekg: o. B.	Daumenspitzen-gross. Besonders stark gerötet.
58	26	♀	Hausangestellte	(Nr. 61/41.) Vor 4 J. fieberhafte Angina. Danach oft, besonders im Winter, Schmerzen im Rachen, im vorigen Winter häufiger als früher. Bei den Rachenschmerzen nur selten Fieber. Neigung zu Schnupfen und Kopfschmerz. Während 1 J. oft wiederkehrende Schmerzen in verschiedenen Gelenken. Hat sich besonders müde gefühlt.	14. 3. 1941. Allgemeinzustand befriedigend.	Daumenspitzen-gross, gerötet, grubig. In beiden Pfröpfe. In den Kieferwinkeln ein paar indolente erbsengrosse Drüsen.
59	22	♀	Arbeiterin	(Nr. 66/41.) Angina zum ersten Male mit 9 J., danach regelmässig mindestens einmal jährlich. Peritonsillarabszesse mehrere Male, zuletzt vor 2 J. Letzte Angina vor 1 Mon. Bekommt leicht Schnupfen und Husten.	21. 3. 1941. O. B.	Pflaumengross, gerötet, grubig.
60	33	♀	Waschfrau	(Nr. 70/41.) Während 7 J. Anginen regelmässig im Frühjahr und Herbst, zuletzt vor $\frac{1}{2}$ J. Ausserdem oft ein paar Tage dauernde Empfindlichkeit im Rachen. In den letzten J. fortgesetzt Müdigkeit und oft Schwindel.	26. 3. 1941. O. B.	Pflaumengross, gerötet, grubig. In beiden flüssiges Sekret. In den Kieferwinkeln ein paar indolente Drüsen.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillektomie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
2 kariöse Zähne.	Nephritis acuta.	7. 2. 1941	In den Veränderungen des Harnes keine Zunahme.	16. 4. 1942. Hat sich ausgezeichnet befunden. Harn: Eiw. $\pm$ (Opalesz.). Sedim.: 0. Blutdr. 135/90.
Einige Zähne fehlen.		14. 2. 1941	Pflaumengross. Beim Spalten wird im Innern beider Tonsillen eine erbsengrosse, Eiter enthaltende Höhle festgestellt.	Am Tage nach der Tonsillektomie Temp. 38.9°. In der Basis der l. Lunge in einem zirkumskripten Gebiet verhärtetes Rasseln. Bekam eine Sulfapyridinkur. Das Fieber und das Rasseln verschwand in 3 T.
Oben eine Prothese. Unten fehlen mehrere Zähne.	Gelenkbeschwerden. Müdigkeit.	18. 3. 1941	In beiden Tonsillen einige erbsengrosse und kleinere Eiterherde.	28. 4. 1941. Symptomlos.
Beiderseits unten 2 kariöse Zähne.		22. 3. 1941	R. Tonsille sehr adhärent, narbig. In beiden kleine Eiterherde.	19. 5. 1941. Symptomlos.
5 Zähne fehlen. 3 kariöse Zähne.	Müdigkeit.	27. 3. 1941	Sehr gross, in beiden kleine Eiterherde.	23. 5. 1941. Befinden nach der Tonsillektomie gut. Weder Müdigkeit noch Schwindel.

Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
61	33	♂	Maschinenmeister	(Nr. 72/41.) 2 J. lang von Zeit zu Zeit Temperaturerhöhung, die etwa je 1 W. anhält. Dabei oft Empfindlichkeit im Rachen, aber keine ausgeprägten Anginen. Während der 2—3 letzten Mon. fast regelmässig abends Temperaturerhöhung (bis 37.4°).	27. 3. 1941. Allgemeinzustand gut. Temp. 37.3°. Röntgenbild des Thorax: o. B.	Klein, gerötet, in beiden Pfröpfe.
62	13	♂	Schüler	(Nr. 77/41.) Ein paar J. lang im Rachen hin und wieder leichte Empfindlichkeit. Fieberhafte Anginen früher einige Male und zuletzt vor 2 W., wo sie 4 T. dauerte. Unmittelbar danach vom einen Gelenk zum anderen wechselnde Schmerzen. Kein Fieber.	2. 4. 1941. Von grazilem Bau, blass. An den beiden Handwurzelgelenken Tastempfindlichkeit. Ekg: o. B.	Daumenspitzen-gross, grubig, in beiden flüssiges Sekret. In der Kieferwinkeln daumenspitzen-grosse, empfindliche Drüsen.
63	30	♀	Viehpflegerin	(Nr. 81/41.) Vom 10. Lebensjahr an Anginen, in den letzten Jahren etwa 2 mal im Winter. 2 mal ein Peritonsillarabszess, zuletzt vor 8 Mon. Letzte Angina vor 3 Mon. 3—4 J. leichte Gelenkschmerzen nach den Anginen regelmässig stärker. Andauernd Müdigkeit.	4. 4. 1941. Allgemeinzustand gut. An den beiden Ellbogengelenken Tastempfindlichkeit.	Gross, gerötet, grubig. Im 1. Kieferwinkel eine bohnen-grosse Drüse.
64	16	♂	Landwirtssohn.	(Nr. 84/41.) Seit dem 8. Lebensjahr Angina 1—2 mal jährlich. Vor 3 J. Peritonsillarabszess. Zuletzt vor 2 Mon. Angina. Danach fast unausgesetzt Empfindlichkeit im Rachen, 2 Mon. lang auch so müde, dass Arbeit unmöglich war.	8. 4. 1941. O. B. Ekg: o. B.	Daumenspitzen-gross, grubig, fest. In beiden Pfröpfe.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillektomie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
O. B.	Temperaturerhöhung.	28. 3. 1941	Pflaumengross, lassen sich leicht ablösen. Makroskopisch sind keine Eiterherde festzustellen. Temp. am Operationstag 37.8°.	
Im Unterkiefer 1 kariöser Molar.	Infectio rheumatica acuta.	3. 4. 1941	Beide Tonsillen sehr gross, in beiden Eiterherde. Gleichzeitig wurde eine Abrasio tonsillae pharyngis ausgeführt.	Nach der Operation verschwanden die Gelenkschmerzen schnell. 6. 6. 1941. Symptomlos. Die Drüsen beinahe verschwunden.
O. B.	Polyarthritidis levis. Müdigkeit.	5. 4. 1941	Klein, nicht adhären. Keine makroskopischen Eiterherde. Nach der Tonsillektomie an 2 T. Temperaturerhöhung auf 37.5°.	13. 3. 1942. Befinden gut. Müdigkeit verschwunden. Keine Gelenksbeschwerden mehr.
2 kariöse Molaren.	Müdigkeit.	9. 4. 1941	Daumenspitzen-gross, leicht adhären. Keine makroskopischen Eiterherde.	16. 6. 1941. Befinden nach der Operation anfangs durchaus gut, bis vor 3 W. eine epidemische Parotitis auftrat, während der 3 T. lang Fieber bestand.

Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
65	19	♂	Lagerarbeiter	(Nr. 90/41.) Vor $\frac{1}{2}$ J. während 1 $\frac{1}{2}$ Mon. 3 mal eine fieberhafte Angina. Danach Rachen ab und zu empfindlich und sehr starke Müdigkeit. Gleichzeitig Neigung zu Herzklopfen.	18. 4. 1941. Allgemeinzustand gut. Beide Lappen der Gl. thyreoidea taubeneigross, fest. Im Isthmus ein daumenspitzengrosser Knoten. Ekg: o. B.	Daumenspitzen-gross, gerötet. In beiden Pfröpfen. In den Kieferwinkeln einige indolente, boh-nengrosse Drü-sen.
66	25	♀	Landwirts-tochter	(Nr. 96/41.) 3 J. Struma, die sich allmählich vergrös-sert hat. Während des letzten Jahres 2 mal fieber-hafte Angina. Im Schlund oft Empfindlichkeit.	27. 4. 1941. Allgemein-zustand gut. R. Lappen der Gl. thyreoidea gän-seeigross, kleinknotig.	Daumenspitzen-gross, besonders die r. grubig.
67	18	♀	Landwirts-tochter	(Nr. 101/41.) 5 J. lang Struma, die sich allmäh-lich vergrössert hat. Be-sonders im Winter oft Emp-findlichkeit im Rachen. Pat. hat keine fieberhaften Anginen gehabt.	26. 4. 1941. Allgemein-zustand gut. In beiden Lappen der Gl. thyreoidea hühnereigrosse, feste Adenome.	Pflaumengross, sehr grubig. Aus beiden kommen Pfröpfe.
68	22	♀	Arbeiter-frau	(Nr. 106/41.) 4 J. lang Struma. Neigung zu Herz-klopfen. 3 mal fieberhafte Angina, zuletzt vor 1 $\frac{1}{2}$ J. Während des letzten Jah-res andauernd Schmerzen im Schlund. Es sind übel-riechende »Graupen« in den Mund aufgestiegen. Fort-während müde. Pat. kann kaum ihre gewöhnlichen Hausarbeiten ausführen. Bekommt leicht Schnupfen.	7. 5. 1941. Allgemein-zustand mässig gut. Blass. Beide Lappen der Gl. thyreoidea hüh-nereigross, kleinknotig. Ekg: o. B.	Pflaumengross, grubig. In bei-den zahlreiche Pfröpfe und flüs-siges Sekret. In den Kieferwin-keln ein paar boh-nengrosse, in-dolente Drüsen.
69	19	♀	Landwirts-tochter	(Nr. 110/41.) Hat von Kind auf jährlich mehrere Angi-nen gehabt, zuletzt vor 4 Mon. Bekommt im allge-meinen leicht Schnupfen und Husten.	12. 5. 1941. O. B.	Ausserordentlich gross, grubig. In beiden Pfröpfe.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillekto- mie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
O. B.	Müdigkeit. Struma nodosa.	19. 4. 1941	Pflaumengross, narbig. In beiden zahlreiche Pfröpfe. Nach der Opera- tion an 3 T. Tempe- raturerhöhung bis 37.5°.	26. 5. 1941. Befinden gut. Arbeitet wieder.
Mehrere kariöse Zähne.	Struma nodosa.	28. 4. 1941	Daumenspitzen-gross, etwas adhären. Keine makroskopischen Eiter- herde. Nach der Opera- tion an 2 T. Tempera- turerhöhung.	13. 3. 1942. Schreibt, dass sie sich wohl be- finde. Kann nicht zur Spät-Nachuntersuchung kommen.
Mehrere kariöse Wurzeln, worin röntgenologisch keine Herde.	Struma nodosa.	3. 5. 1941	Beide Tonsillen gross. Makroskopisch o. B. Nach der Operation 3 T. Temperaturerhöhung auf 37.5°.	
4 kariöse Zähne.	Müdigkeit Struma nodosa.	8. 5. 1941	Pflaumengross. Der obere Lappen der r. adhären. In beiden kleine Eiterherde und feste Stellen. Nach der Operation an 2 T. Tem- peraturerhöhung bis 37.4°.	16. 3. 1942. Befinden gut gewesen. Müdigkeit verschwunden. Jetzt eine Woche Schnupfen. Immer noch Neigung zu Herzklopfen.
2 kariöse Zähne.		13. 5. 1941	Beide Tonsillen sehr gross, in beiden Pfröpfe.	16. 3. 1942. Das Befin- den nach der Operation durchaus gut.



Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
70	42	♀	Schofförs- witwe	(Nr. 112/41.) Letzter Partus vor 12 J. Danach im ganzen 5 Aborte, zuletzt vor 2 J., wo im Zusammenhang mit dem Abort eine einige W. dauernde Adnexitis, die später als geheilt nachgewiesen wurde. Anginen in jüngeren J. dann und wann, während der letzten 3 J. 2—3 mal. Angina vor 2 Mon. und zuletzt vor 2 W., wonach Rachen andauernd empfindlich. Etwa 2 Mon. ausserordentlich müde und schwach. In den Handwurzeln und Ellbogengelenken fortgesetzt Schmerzen.	15. 5. 1941. Mager und blass. In beiden Lappen der Gl. thyreoidea ein paar mandel- bis pflaumengrosse Adenome. Gynäkol. Unters.: o. B.	Tonsillen tief in den Gaumenbögen, gerötet, grubig. In der l. sind Pfröpfe. Auf der Innenfläche der Mandibula und in beiden Kieferwinkeln einige bohnen-grosse, etwas schmerzhaft Drüsen.
71	24	♀	Viehpflegerin	(Nr. 113/41.) Seit dem 16. J. Anginen mindestens einmal jährlich. Vor 5 Mon. ein Peritonsillarabszess. Letzte Angina vor 1 ½ Mon. Ein paar J. lang nach den Anginen regelmässig einige W. dauernde Gelenkschmerzen. Bei der Aufnahme in die Klinik keine Gelenksbeschwerden.	16. 5. 1941. Allgemeinzustand gut. Beide Lappen der Gl. thyreoidea leicht vergrössert. In ihrem Innern kleine Knoten.	Daumenspitzen-gross, gerötet, ödematös. In beiden flüssiges Sekret.
72	33	♀	Bauersfrau	(Nr. 117/41.) Vor 10 J. Angina. Danach Herzbeschwerden. War 6 W. bettlägerig. Nach Angabe des Arztes handelte es sich um eine Karditis. Nachträglich keine anderen Symptome als zeitweise Neigung zu Herzklopfen. Während der letzten 7 Mon. 3 mal Angina, zuletzt vor 5 W. Danach im Schlund fortgesetzt Schmerzen. Pat. war sehr müde.	21. 5. 1941. Allgemeinzustand gut. Klang der Herztöne leicht herabgesetzt. Ekg: o. B. In den Fäzes Bothriophalus-Eier.	Fingerspitzen-gross, gerötet, grubig. In beiden Pfröpfe. Im l. Kieferwinkel eine bohnen-grosse, leicht empfindliche Drüse.
73	25	♂	Arbeiter	(Nr. 118/41.) Hat während 5 J. mehrere Anginen im J. gehabt. 1 J. lang Rachen ab und zu empfindlich und oft wiederkehrende Schmerzen in den Gliedern. 1 Mon. Schmerzen besonders im Kreuz. Bewegungen infolgedessen erschwert.	23. 5. 1941. Allgemeinzustand gut. Lasègue rechts + etwa 60°. Das Röntgenbild des Kreuzes: o. B.	Daumenspitzen-gross, stark gerötet, grubig. In beiden Sekret.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillekto- mie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
Oben eine Prothese. Unten fehlen mehrere Zähne. 2 kariöse Wurzeln.	Struma nodosa. Gelenkbeschwerden.	16. 5. 1941	Reichlich daumenspitzen-gross, in beiden zahlreichen kleine Eiterherde. Am folgenden Tage Temp. 37.6° danach afebril.	13. 3. 1942. Symptomlos. Allgemeinzustand bedeutend gebessert. 2 T. lang Schnupfen.
5 kariöse Zähne.	Struma nodosa.	17. 5. 1941	Pflaumengross. In beiden zahlreiche Eiterherde.	Sofort nach der Operation begann die Temp. zu steigen, 2 T. später Temp. 40° und in der Basis der l. Lunge ein pneumonischer Herd. Pat. bekam eine Sulfapyridinkur, wobei das Fieber und die klinischen Veränderungen in 3 T. verschwanden. 12. 6. 1941. R-Durchleuchtung: o. B.
Mehrere Zähne fehlen.	Müdigkeit. Helminthiasis (Bothriocephalus).	23. 5. 1941	Daumenspitzen-gross, voll Pfröpfe. Am Tage nach der Operation Temp. 37.3°.	3 T. nach der Tonsillektomie an 2 T. Temp. 38° und Schnupfeninfektion. Beläge auf den Wundflächen grösser als gewöhnlich. 8 T. nach der Tonsillektomie erfolgreiche Wurmkur. 19. 3. 1942. Befinden nach der Operation gut. Zugenommen. 3 T. Schnupfen.
Mehrere Zähne fehlen. 4 kariös.	Syndroma ischiadicum.	24. 5. 1941	Makroskopisch keine Veränderungen. Nach der Operation an 4 T. Temperatur bis 37.6°.	4. 6. 1941. Bei der Entlassung aus dem Krankenhaus symptomlos.

Tabelle  
Klinische Angaben über die

Nr.	Alter	Geschlecht	Beruf	Anamnese	Allgemeinzustand	Tonsillenstatus
74	33	♀	Versicherungsbeamtin	(Nr. 122/41.) In früheren Jahren mitunter Angina. Während der letzten 8 Mon. 3 mal Angina, zuletzt vor über 1 Mon., wonach im Rachen fast un- ausgesetzt Schmerzen und andauernd sehr müde. 2 J. lang oft wiederkehrende Schmerzen in den Gelenken. Im Zusammenhang mit den letzten Anginen verschlimmerten sich die Gelenkbeschwerden.	27. 5. 1941. O. B.	Daumenspitzen-gross, gerötet, grubig. In beiden flüssiges Sekret. In den Kieferwinkeln ein paar indolente Drüsen.
75	25	♀	Bauersfrau	(Nr. 130/41.) Von Kindheit an mehrere fieberhafte Anginen im J., zuletzt vor etwa 1 Mon. Nach der letzten Angina wechselnde Schmerzen in den Gelenken.	4. 6. 1941. O. B.	Walnussgross, gerötet. In beiden Pfröpfe.
76	21	♀	Landwirts- tochter	(Nr. 133/41.) In den letzten Jahren besonders im Winter oft Empfindlichkeit im Schlunde und in Verbindung damit eine kleine Temperaturerhöhung. Andauernd sehr müde.	10. 6. 1941. Allgemein-zustand mässig gut.	Tonsillen klein, gerötet. Aus der rechten kommt flüssiges Sekret.
77	55	♀	Arbeiterfrau	(Nr. 137/41.) Von Jugend auf Anginen, während der letzten 6 J. mehrmals im J. Rachen während des letzten Winters ab und zu empfindlich und im Zusammenhang damit oft Temperaturerhöhung. 4—5 J. (nach dem Klimakterium) in den Knien oft Schmerzen. In den letzten Mon. Zustand der Knie schlechter als vorher und auch in den anderen Gelenken von Zeit zu Zeit Schmerzen.	14. 6. 1941. Allgemein-zustand gut. In beiden Knien Krepitation bei Bewegungen, sonst Gelenke o. B.	Daumenspitzen-gross, kein Sekret. In den Kieferwinkeln ein paar indolente Drüsen.

## 21 (Forts.).

## Tonsillitis chronica-Fälle.

Zahnstatus	Krankheiten oder Symptome ausser der Tonsillitis	Zeitpunkt der Tonsillekto- mie	Operationsbefunde über die Tonsillen, Fieber oder andere Reaktionen unmittelbar nach der Operation	Decursus. Spät-Nachuntersuchung
Abgestorbene Zähne: 6 +, 5 +, 1 +, 8 —, 5 — und —7. Rönt- genologisch sind keine periapika- len Veränderun- gen festzustellen.	Gelenkbeschwer- den. Müdigkeit.	28. 5. 1941	Daumenspitzen-gross, in beiden zahlreiche Pfröp- fe. Nach der Operation Temp. an 2 T. 37.3°.	18. 3. 1942. Befinden gut.
Keine kariösen Zähne. Einige Plomben.	Gelenkbeschwer- den.	5. 6. 1941	Walnussgross. Makro- skopisch keine Eiter- herde zu konstatieren. Am Tage nach der Ope- ration Temp. 37.4°.	20. 3. 1942. Laut brief- licher Mitteilung fühlt sich Pat. wohl. Kann nicht zur Spät-Nach- untersuchung kommen.
Zähne gepflegt. In einem Wur- zelfüllung.	Müdigkeit.	11. 6. 1941	In der r. Tonsille eine korn-grosse, Eiter ent- haltende Höhle. Die l. ein wenig adhärent.	17. 3. 1942. Befinden gut.
Mehrere Zähne fehlen. 2 kariöse Zähne.	Arthritis chro- nica genuum.	16. 6. 1941	Tonsillen walnussgross, die rechte stark adhä- rent. In ihrem Innern Eiterherde.	13. 3. 1942. Immer noch Schmerzen in der Knien, aber weniger als früher. In den übrigen Gelenken keine Sym- ptome. Allgemeinbefin- den bedeutend besser als vor der Tonsillekto- mie.

## Das klinische Krankheitsbild.

Bei der Sammlung der Tonsillitispatienten wurde eine gewisse Auswahl hinsichtlich der Fälle vorgenommen. Ich habe mich bemüht, solche Fälle zu vermeiden, in denen ausser der Tonsillitis weitentwickelte sogenannte Sekundärkrankheiten vorlagen, die Veränderungen im Sternalpunktat und im Blutbild hervorrufen konnten. Doch klagten die meisten Patienten ausser über direkt von den Tonsillen herstammende örtliche Symptome auch über gewisse Beschwerden oder es waren bei ihnen andere leichte Krankheiten zu konstatieren. Die Anzahl und Natur der Begleitkrankheiten oder -symptome geht aus dem folgenden Verzeichnis hervor:

	{ Ohne subjektive Symptome . . . . .				waren 6 Patienten
	{ Müdigkeit . . . . .	lag	vor	bei 16	»
	{ Rheumatische Beschwerden . . . .	lagen	vor	» 19	»
I	{ Nephritis . . . . .	lag	vor	» 2	»
	{ Herzfehler . . . . .	»	»	» 2	»
	{ Asthma bronchiale . . . . .	»	»	» 1	»
	{ Dauernde Temperaturerhöhung	»	»	» 1	»
	{ Struma adenomatosa non toxica	»	»	» 8	»
	{ Helminthiasis (Bothr. latus) . .	»	»	» 6	»
II	{ Anaemia hypochromica essen-				
	{ tialis . . . . .	»	»	» 1	»
	{ Kachexia hypophysaria . . . . .	»	»	» 1	»

In diesem Verzeichnis fehlen noch die an den Zähnen festgestellten Krankheiten, die später besprechen werden. Die Krankheiten oder Symptome sind getrennt verzeichnet je nachdem, ob sie sekundär von einem Tonsillenherd ausgegangen sein können (I) oder ob sie Nebenkrankheiten sind, die keinen ätiologischen Zusammenhang mit der Tonsillitis haben dürften (II). Den tonsillo-genen Ursprung der ersteren können wir auf Grund der Anamnese und der Nachuntersuchung mit mehr oder weniger grosser Wahrscheinlichkeit annehmen, aber wir können ihn natürlich nicht mit voller Sicherheit nachweisen.

Müdigkeit, worüber manche ( $\frac{1}{3}$ ) meiner Patienten ausschliesslich klagen, kommt selbstverständlich als Symptom bei allerlei



Angina eingestellt hatte. Bei einem Patienten (61) trat als Sekundärkrankheit ein andauernder Temperaturanstieg auf. Bei diesem waren die anamnestischen Tonsillitis-symptome leicht, und auch bei der Operation waren keine makroskopischen entzündlichen Veränderungen zu konstatieren, weshalb es unklar bleibt, ob die Tonsillitis die fortgesetzte Subfebrilität veranlasst hatte.

Im ganzen genommen, bildet mein Material eine fortlaufende Reihe von Fällen, bei denen subjektiv und objektiv ausser der Tonsillitis keine anderen Krankheiten auftreten, bis zu Patienten mit subjektiven Beschwerden und zuletzt bis zu Fällen, bei denen auch objektiv feststellbare leichte Sekundärkrankheiten vorliegen.

### Die Tonsillenbefunde.

Die chronische Tonsillitis ist ein Begriff, der sowohl klinisch als pathologisch-anatomisch schwer genau abzugrenzen ist. Die Grenze zwischen der gesunden und der chronisch entzündeten Tonsille ist nicht klar. Darum ist es in diesem Zusammenhang angebracht, die Tonsillenbefunde bei meinen Patienten genauer zu betrachten und nachzusehen, inwieweit die Tonsillitisdiagnose auf Grund derselben als sicher betrachtet werden darf.

Die Diagnose Tonsillitis chronica können wir auf Grund der Anamnese, der klinischen, der histologischen und der zytologischen Untersuchung des aus den Tonsillen erhaltenen Sekretes stellen. Schon aus der Anamnese lassen sich wichtige Schlussfolgerungen auf den Zustand der Tonsillen ziehen. »Der sicherste Beweis für eine chronische Tonsillitis ist immer noch das Vorkommen rezidivierender Anginen« (HAJEK). Mit Hilfe histologischer Untersuchungen konnte nachgewiesen werden, dass in den Tonsillen von Personen, die rezidivierende Anginen gehabt haben, in den Intervallen zwischen diesen auf eine andauernde chronische Entzündung hinweisende Veränderungen bestehen. Die Fälle, in deren Anamnese Anginen vorliegen, histologisch aber keine entzündlichen Veränderungen in den Tonsillen festzustellen sind, bilden Ausnahmen (HOLSTI). Von besonderer Bedeutung sind in der Ätiologie der chronischen Tonsillitis frühere peritonsilläre Abszesse, da nach





sind u. a. KÄHLER und RENN gelangt, nach denen das Sekret bei Patienten mit chronischer Tonsillitis leukozytenhaltig ist, aber keine Leukozyten enthält, wenn keine Entzündung besteht.

Wie die allgemeine Erfahrung lehrt, gibt es zahlreiche Fälle, in denen in den Tonsillen schwere entzündliche Veränderungen vorliegen, obgleich bei der Inspektion und der zytologischen Untersuchung des Sekretes keine pathologischen Symptome nachgewiesen werden konnten. Das dürfte auf den sich in den Krypten bildenden Blindsäcken beruhen, in denen der Entzündungsprozess okkult bleibt. Erst durch eine histologische Untersuchung erhalten wir ein genaues Bild von dem tatsächlichen Zustand der Tonsillen.

In meinen Fällen wurde die Diagnose chronische Tonsillitis auf Grund der Anamnese und der Inspektion der Tonsillen gestellt. Eine histologische Prüfung und eine zytologische Untersuchung des Tonsillensekretes wurden nicht ausgeführt. Die bei der Operation vorgenommene makroskopische Besichtigung der Tonsillen stellt in manchen meiner Fälle die Diagnose Tonsillitis sicher. Bei der Operation selbst treten Verwachsungen der Mandeln mit der Umgebung hervor. Ferner gewinnt man eine bessere Auffassung von der Konsistenz der Tonsillen. Ihre Spaltung und Zerstückelung enthüllt intratonsilläre Abszesse und Narbenbildungen, die durch blosse Inspektion nicht zur Beobachtung kommen. Indessen ist es klar, dass die Entzündung nicht immer bei der makroskopischen Untersuchung zum Vorschein kommt, obwohl sie vorhanden ist.

Die Tonsillenbefunde meiner Patienten ergeben sich aus der folgenden Zusammenstellung:

In der Anamnese fanden sich rezidivierende		
Anginen .....	in 35 Fällen	
Unter diesen war der Operationsbefund positiv .....	in 22	»
unsicher .....	in 4	»
negativ oder fehlte .....	in 9	»
In der Anamnese fand sich nur einmal Angina		
oder leichte Schlingbeschwerden ..	in 10	»
Unter diesen war der Operationsbefund positiv .....	in 6	»
negativ oder fehlte .....	in 4	»

Wie aus diesem Verzeichnis hervorgeht, gründete sich die Diagnose chronische Tonsillitis entweder auf eine deutlich positive Anamnese oder einen positiven Operationsbefund oder auf beide bei allen Patienten ausser vier (33, 41, 61 und 67). Bei den letzteren traten anamnestisch nur leichte Symptome auf, und der makroskopische Operationsbefund war negativ. In diesen Fällen gründete sich die Diagnose Tonsillitis hauptsächlich auf die bei der Inspektion gemachten Beobachtungen.

### Die Zähne.

Die Angaben über den Zahnstatus in Tabelle 21 gewähren eine summarische Auffassung vom Zustand der Zähne bei den Patienten. In 4 Fällen (39, 48, 51 und 53) bestanden schwere kariöse Veränderungen, und in 2 Fällen (49 und 55) wurde röntgenologisch eine Ostitis periapicalis festgestellt. Bei diesen Patienten schien eine Zahnsanierung notwendig. Von der möglichen Einwirkung der Zahninfektion und -extraktion auf meine Ergebnisse werde ich später sprechen. In den übrigen Fällen wurden bei 3 Patienten Zahn-Röntgenuntersuchungen ausgeführt, die keine periapikalen Veränderungen erkennen liessen. Ausserdem trat noch bei 18 Patienten Karies auf, jedoch in mässigem Grade. Auch diese Patienten konnten Infektionsherde an ihren Zähnen haben, aber diese Möglichkeit ist nicht gross.

### Die Spät-Nachuntersuchungen.

Es war meine Absicht, bei jedem Patienten frühestens einen Monat nach der Tonsillektomie eine Spät-Nachuntersuchung auszuführen. Waren die Patienten meiner Aufforderung, sich zur Spät-Nachuntersuchung einzustellen, nicht nachgekommen, so schickte ich noch einen Brief, in dem ich an die Sache erinnerte. Jedoch kamen nur 30 Patienten, also  $\frac{2}{3}$  meines Materials, zu der Spät-Nachuntersuchung. Ausserdem teilten 3 Patienten brieflich mit, dass sie sich wohl befänden. Dass sich  $\frac{1}{3}$  der Patienten nicht zu der Spät-Nachuntersuchung einfand, erklärt sich grossenteils aus den ungünstigen Verhältnissen zwischen den zwei Kriegen, unter denen ich meine Arbeit ausführen musste.

Da es dem Leser schwer werden wird, aus der die klinischen Daten meiner Patienten enthaltenden Tabelle 21 getrennt die



Tabelle 22 (Forts.).  
Die zur Spät-Nachuntersuchung gekommenen Patienten.

Nr.	Tonsillen- anamnese <sup>1</sup>	Operations- befund	Begleit- und andere Krank- heiten oder -symptome neben der Tonsillitis	Zähne	Zeitpunkt der Spät-Nachunter- suchung	Begleitkrankheiten oder -symptome bei der Spät-Nachunter- suchung
54	+	+	Müdigkeit	1 kariöser Zahn	2 ½ Mon. nach der Tonsillekt.	Symptomlos
55	+	+	Nephritis ac. Helminthiasis	Ostitis periapi- cal. (Zähne nach d. Tonsillekt. extrahiert)	13 Mon. nach der Tonsillekt.	Besser
56	+	Fehlt	Nephritis ac.	2 kariöse Zähne	2 ¼ Mon. nach der Tonsillekt.	"
58	+	+	Müdigkeit, Gelenkbeschw.	O. B.	1 ¼ Mon. nach der Tonsillekt.	Symptomlos
59	++	+		4 kariöse Zähne	1 ¾ Mon. nach der Tonsillekt.	"
60	+	+	Müdigkeit	3 kariöse Zähne	2 Mon. nach der Tonsillekt.	"
62	+	+	Inf. rheum. ac.	1 kariöser Zahn	2 Mon. nach der Tonsillekt.	"
63	++	--	Polyarthr. chr. Müdigkeit	O. B.	11 Mon. nach der Tonsillekt.	"
64	++	+	Müdigkeit	2 kariöse Zähne	2 ¼ Mon. nach der Tonsillekt.	Symptomlos Convalescentia post parotitidem
65	+	+	Müdigkeit Struma nodosa	O. B.	1 ¼ Mon. nach der Tonsillekt.	Symptomlos
68	+		Müdigkeit Struma nodosa	4 kariöse Zähne	10 ¼ Mon. nach der Tonsillekt.	"
69	+	±		2 kariöse Zähne	10 Mon. nach der Tonsillekt.	Symptomlos ausser einer zufäll. akut. Inf.
70	+	+	Gelenkbeschw. Struma nodosa	2 kariöse Zähne	10 Mon. nach der Tonsillekt.	Symptomlos ausser einer zufäll. akut. Inf.
72	+	±	Müdigkeit Helminthiasis	O. B.	10 Mon. nach der Tonsillekt.	Symptomlos ausser einer zufäll. akut. Inf.
74	+	+	Gelenkbeschw. Müdigkeit	Wurzelfüllungen Röntgen: O. B.	9 ½ Mon. nach der Tonsillekt.	Symptomlos
76	±	+	Müdigkeit	1 Wurzelfüllung	9 Mon. nach der Tonsillekt.	"
77	+	+	Arthr. chr. genuum	2 kariöse Zähne	9 Mon. nach der Tonsillekt.	Besser

<sup>1</sup> ± = in der Anamnese keine deutlichen Anginen

+ = " " " eine oder mehrere "

++ = " " " " " " " und Peritonsillarabszesse

Tabelle 23.

Sekundärkrankheiten oder -symptome vor der Tonsillektomie	Zahl der Fälle	Sekundärkrankheiten oder -symptome bei der Spät-Nachuntersuchung			Bemerkungen
		Sym- ptomlos	Besser	Wie vorher	
Ohne Sekundärkrankheiten	2	2	—	—	Bei der Spät-Nachuntersuchung 1 mit akuter Infektion
Müdigkeit	12	12	—	—	Bei der Spät-Nachuntersuchung 2 mit akuter Infektion.
Rheumatische Schmerzen ohne obj. nachweisbare Veränderungen	6	6	—	—	Bei der Spät-Nachuntersuchung 1 mit akuter Infektion.
Polyarthrititis	7	5	2	—	
Nephritis	2	—	2	—	
Asthma bronchiale	1	1			

Angaben über die der Spät-Nachuntersuchung unterworfenen Fälle herauszulesen, sind sie ausserdem gesondert in Tabelle 22 kurz zusammengestellt, obgleich sich dadurch Wiederholungen ergeben haben.

Die kürzeste Zeit zwischen der Tonsillektomie und der Spät-Nachuntersuchung beträgt  $1\frac{1}{4}$  Monat und die längste  $18\frac{1}{2}$  Monate. Durchschnittlich wurde die Spät-Nachuntersuchung 5.9 Monate nach der Tonsillektomie vorgenommen. Tabelle 23 gibt ein Bild von den zu der Spät-Nachuntersuchung erschienenen Patienten, eingeteilt in verschiedene Gruppen je nach von den Sekundärkrankheiten und -symptomen.

Aus der Tabelle geht hervor, dass, abgesehen von 4 Fällen, die Sekundärkrankheiten oder -symptome bei der Spät-Nachuntersuchung verschwunden waren. Wahrscheinlich hat diese günstige Wirkung mit der Tonsillektomie und in 5 Fällen auch mit der gleichzeitigen Entfernung der Zahnherde in Zusammenhang gestanden. Bei der Spät-Nachuntersuchung zeigten 3 Patienten eine zufällige Rhinopharyngitisinfektion, und einer war unlängst von einer epidemischen Parotitis genesen.

## b) Die Sternalpunktate.

Die Sternalpunktionen, die teils vor, teils nach der Tonsillektomie ausgeführt wurden, zusammen 146, sind in Tabelle 24 zusammengestellt.

## Vor der Tonsillektomie.

Der Zellenreichtum war in den vor der Tonsillektomie entnommenen Punktaten ähnlicher Art wie bei den Normalfällen. Der Erythroblasten-Leukozytenindex war durchschnittlich 1:5. In den Korrelationen der Zellen waren mehrere einzelne vom Normalen abweichende Werte zu bemerken. Eine allgemeine Auffassung gewinnen wir, wenn wir die Mittelwerte der vor der Tonsillektomie entnommenen Punktate mit den normalen Mittelwerten (Tabelle 25) vergleichen. Im folgenden betrachte ich die Mittelwerte der verschiedenen Zellarten Gruppe für Gruppe. Die Mengen der basophilen und der eosinophilen Zellen weichen nicht nennenswert voneinander ab. Prototypen der Neutrophilen von den Myeloblasten an (jedoch mit Ausschluss der Myelozyten) bis zu den Stabkernigen kommen bei den Tonsillitispatienten durchschnittlich etwas mehr vor als bei den Normalfällen. Prototypen fanden sich bei den Patienten zusammen durchschnittlich 54 % und in den Normalfällen 51 %. Es handelt sich also nicht um einen grossen Unterschied. Eine erheblichere Differenz tritt dagegen hinsichtlich der segmentkernigen Neutrophilen auf. Bei den Normalfällen kamen solche im Mittel in 29 % vor und bei den Tonsillitispatienten in 19 %. Abbildung 26 veranschaulicht graphisch die Variationsgrenzen der Segmentkernigen. Daraus wird ersichtlich, dass von den Werten der Tonsillitispatienten nur  $\frac{1}{3}$  innerhalb der Variationsgrenzen der Normalfälle fällt, während  $\frac{2}{3}$  der Fälle mit ihren Werten unterhalb der unteren Grenze der Normalfälle liegen. Niedrige Werte der Segmentkernigen sind also bei meinen Patienten gewöhnlich.

In der Gruppe der Lymphozyten besteht gleichfalls ein deutlicher Unterschied zwischen den Patienten und den Normalfällen. Die Patienten wiesen durchschnittlich 21 % und die Normalfälle 14 % Lymphozyten auf. Wie bei den Neutrophilen beruht die Abweichung von der Norm auch in diesem Fall nicht auf ein-

Tabelle 24.  
Die Sternalpunkte der Tonsillitis chronica-Fälle.

Nr.	Datum der Tonsillektomie	Datum	%										Zahl auf 400 L						Retikuloz.		%
			Bas. Myeloz. & Leukoz.	Eos. Myeloz.	Eos. Leukoz.	Myelobl.	Neutr. Promyeloz.	Neutr. Myeloz.	Neutr. Metamyeloz.	Neutr. stabk. Leukoz.	Neutr. segm. Leukoz.	Lymphoz.	Monoz.	Retikulum- & Plasmaz.	Megakar.	Normobl.	Makrobl.	Proerythrobl.	Rote Mitoser	Weisse Mitosen	
33	19/4 — 39	18/4 — 39	0	4.00	1.50	1.25	4.50	16.25	23.50	12.50	13.25	23.25	0	15	0	50	5	2	1	2	
		20/4 »	0.25	2.50	1.25	0.50	1.75	7.25	10.00	6.50	34.50	35.25	0.25	11	0	12	0	0	0	0	
		25/4 »	0.25	0.75	1.75	1.00	3.00	13.25	23.50	10.00	17.00	29.50	0	17	0	34	2	0	0	1	
34	29/4 — 39	26/4 — 39	0	0.75	0	1.25	3.75	13.25	24.25	10.00	24.50	21.75	0.50	10	0	99	10	3	0	2	
		2/5	0	2.25	0.75	1.50	6.75	10.50	17.50	11.0	20.75	28.25	0.75	3	0	43	3	1	1	1	
35	13/5 — 39	10/5 — 39	0	5.00	1.75	2.75	7.25	10.75	14.50	14.25	15.50	27.50	0.75	25	1	143	24	12	4	5	0.6
		14/5 »	0	3.00	1.75	2.00	5.25	12.75	19.75	12.50	13.75	28.50	0.75	12	0	105	16	7	4	0	
		19/5 »	0	1.75	2.00	0.75	4.00	9.75	16.50	9.75	25.00	30.50	0	6	1	45	5	0	1	1	
		25/8 »	0	7.00	2.00	1.00	5.75	10.25	15.50	11.50	17.75	26.75	2.50	12	0	100	5	1	2	2	
36	24/5 — 39	20/5 — 39	0.25	8.50	2.75	1.50	5.25	12.00	20.75	10.50	18.25	19.50	0.75	8	0	39	7	0	0	0	
		25/5 »	0	5.25	1.50	1.75	9.25	10.50	22.25	10.25	19.50	19.25	0.50	6	1	56	3	2	1	0	
		13/6 »	0.25	5.00	3.25	0.75	2.50	11.75	15.00	11.50	23.50	26.00	0.50	3	1	43	2	1	3	0	0.5
		9/12 — 40	0.25	3.50	1.00	1.25	4.00	17.25	18.25	12.00	19.00	23.00	0.50	7	0	43	5	0	1	0	
37	10/6 — 39	9/6 — 39	0.75	0.75	1.50	0.25	3.25	7.00	5.00	11.00	36.75	32.25	0.50	2	0	11	1	0	0	0	
		11/6 »	0.25	1.25	2.00	0	3.75	13.75	15.00	11.75	25.50	26.25	0.50	6	0	14	0	0	0	1	
38	10/6 — 39	9/6 — 39	0	4.75	3.75	3.00	7.50	19.75	21.50	13.00	9.25	17.00	0.50	19	1	138	15	4	4	3	
		11/6 »	0.50	6.25	1.00	2.00	6.75	27.00	14.00	12.00	14.25	15.50	0.75	9	0	93	10	0	3	2	
39	30/6 — 39	29/6 — 39	0	2.00	2.25	2.75	7.00	11.00	21.25	19.25	19.25	14.75	0.50	9	1	51	3	0	0	0	1.2
		1/7 »	0.25	3.00	1.75	2.75	9.00	9.75	25.00	16.00	18.25	14.25	0	5	0	44	5	0	0	0	1.2
		10/7 »	0.75	1.75	3.00	1.25	2.75	9.00	15.25	11.75	19.00	35.00	0	4	0	26	10	0	0	0	1.4
		4/8 »	0	2.75	1.75	3.75	4.50	11.25	18.75	12.25	23.75	19.75	1.50	3	1	68	14	0	1	1	

40	8/7 — 39	7/7 — 39 10/7 » 14/8 »	0.75 0 0	1.25 0.50 1.00	1.00 0.25 0.25	2.50 2.50 1.00	7.50 7.75 4.25	12.50 10.50 9.00	23.00 24.50 19.50	13.75 14.50 13.75	23.75 23.00 33.25	14.00 16.50 18.00	0 0 0	2 5 2	0 0 0	64 29 32	4 5 4	0 0 0	2 2 0	2 0 0	1.5
41	17/8 — 39	17/8 — 39 18/8 » 31/8 » 24/11 »	0.75 0.75 0.25 0.50	2.75 1.75 6.00 2.25	1.75 2.75 3.00 2.25	2.75 1.25 3.75 1.50	10.50 8.25 7.00 4.25	20.25 13.00 12.00 11.75	28.00 19.25 19.75 16.25	10.00 7.25 13.25 12.75	8.25 26.25 18.00 28.00	14.75 19.50 14.50 20.00	0.25 0.50 2.50 1.00	7 1 9 6	1 0 2 0	93 26 139 96	33 6 22 14	0 0 0 0	1 0 1 1	4 1 2 3	2.3 1.1 1.8
42	22/8 — 39	21/8 — 39 23/8 »	0 0	2.50 0.75	0.25 0.75	0.75 1.25	1.75 4.50	8.25 6.25	11.75 9.50	6.75 7.00	27.00 44.75	37.00 23.00	4.0 1.75	12 5	0 0	43 21	3 4	0 0	1 0	0 0	0.6
43	27/6 — 10	27/6 — 10 28/6 » 8/7 » 9/8 »	0.75 0 0.50 0.75	2.25 1.25 2.50 1.25	2.00 2.00 0.50 0.25	0.75 2.00 2.00 1.00	4.50 3.50 5.75 3.25	9.25 10.00 12.75 9.50	11.75 15.25 20.75 15.75	10.25 12.00 17.25 12.00	29.50 27.25 22.25 34.50	26.75 24.50 15.25 21.00	2.25 2.25 0.50 0.75	4 3 6 3	0 0 1 1	60 31 65 29	16 10 9 7	0 0 0 0	1 1 2 0	3 0 0 0	0.2 0.8 0.4 0.4
44	2/7 — 10	2/7 — 10 3/7 » 17/2 — 41	0.50 0 0	5.25 6.50 2.75	0.75 3.25 1.00	1.25 2.25 2.25	7.00 10.75 5.00	9.00 16.50 11.75	16.75 15.00 16.00	8.25 9.25 10.00	27.50 16.75 26.00	22.25 19.75 23.50	1.50 0 1.75	8 13 7	1 0 0	81 127 55	10 13 2	0 0 0	0 5 1	1 2 0	2.4
45	9/7 — 10	8/7 — 10 10/7 » 15/4 — 41	0.25 0 0	1.25 2.00 3.25	0.75 1.75 2.00	1.00 1.50 1.25	5.75 2.00 7.75	16.75 8.25 19.50	21.25 4.75 18.25	17.00 8.00 9.25	20.00 45.50 19.00	15.75 24.50 17.75	0.25 1.75 2.00	1 13 6	1 0 0	52 19 83	3 2 4	0 0 1	0 0 1	1 0 1	0.6
46	19/7 — 10	18/7 — 10 20/7 » 29/7 »	0 0 0.25	4.25 2.00 3.00	1.00 0.25 0.50	3.50 0.50 1.25	11.50 8.50 12.50	21.25 20.50 20.00	14.50 21.25 16.25	15.50 16.75 17.00	9.00 12.00 6.75	20.00 18.25 22.25	0.50 0 0.25	9 7 3	0 0 0	73 69 59	7 3 4	1 0 3	1 0 0	1 0 0	2.6
47	30/7 — 10	29/7 — 10 31/7 » 9/8 »	0 0.25 0.75	5.50 2.75 3.75	1.00 1.00 3.50	1.75 1.00 0.50	9.00 6.75 2.00	17.00 16.25 11.00	27.00 22.00 18.00	15.25 13.25 17.50	6.25 18.50 31.25	16.75 18.00 21.25	0.50 0.25 0.50	15 2 11	0 0 0	91 58 13	21 6 2	2 0 0	4 0 0	3 0 0	4.1 1.5 1.8
48	15/10 — 10	14/10 — 10 16/10 » 19/10 » 21/1 — 41	0.50 0.25 0.25 0.50	2.50 3.25 3.25 0.75	1.25 1.00 1.00 1.25	2.50 2.50 0.75 2.00	6.00 5.50 4.50 3.25	13.50 14.50 16.75 13.50	17.75 16.50 24.50 18.00	15.25 14.25 18.00 16.00	17.25 19.00 14.75 15.25	21.75 24.50 15.50 26.50	1.75 1.75 0.75 3.00	4 7 8 14	0 1 0 0	71 111 79 71	8 6 5 5	0 1 1 1	2 2 0 0	0 1 2 0	3.6 1.4 1.4 1.0



Tabelle 24 (Forts.).  
Die Sternalpunkte der Tonsillitis chronica-Fälle.

Die Sternalpunkte der Tonsillus chronica

Nr.	Datum der Tonsillektomie	Datum	%										Zahl auf 400 L						Retikuloz.	Weisse Mitosen	Rote Mitosen	Proerythrobl.	Makrobl.	Normobl.	Megakar.	Retikulum- & Plasmaz.	Monoz.	Lymphoz.	Neutr. segm. Leukoz.	Neutr. stabk. Leukoz.	Neutr. Metamyeloz.	Neutr. Myeloz.	Neutr. Promyeloz.	Myelobl.	Eos. Leukoz.	Eos. Myeloz.	Bas. Myeloz. & Leukoz.

54	9/1 -- 11	8/1 -- 11	0	2.00	0	1.50	21.75	21.75	9.50	15.75	21.50	1.75	13	1	82	12	0	0	0	2.7
		11/1 ,	0	3.50	0.75	1.25	16.25	17.00	11.25	16.00	23.75	2.75	23	0	62	8	1	1	1	2.9
		26/3 ,	0	3.50	0.50	1.25	19.00	21.50	11.75	21.50	12.50	1.25	7	0	32	2	0	0	0	1.1
55	7/2 -- 11	5/2 -- 11	0.50	3.00	1.50	0.50	8.50	17.25	8.25	37.50	20.00	1.25	3	1	88	2	0	1	1	2.6
		11/2 ,	0.25	3.00	3.75	0.25	7.75	8.00	5.75	39.25	29.75	1.25	3	0	26	0	0	0	0	2.5
		10/5 ,	0	2.00	1.00	1.25	16.25	18.50	17.25	21.75	11.25	0	0	0	37	2	0	0	0	0.1
		16/3 -- 12	0	0.75	0.75	0.50	10.00	8.75	15.00	33.50	21.75	1.75	13	0	31	3	0	0	0	0.9
56	7/2 -- 11	5/2 -- 11	0	2.75	1.00	1.75	4.75	21.00	22.50	11.50	17.75	2.50	2	1	58	1	0	0	1	2.8
		11/2 ,	0	3.00	1.25	1.50	18.25	20.50	10.25	17.50	20.50	3.25	11	0	108	11	0	1	0	2.1
		16/4 ,	0	3.75	1.75	1.50	17.00	19.50	11.25	11.00	18.50	1.75	6	0	85	5	1	0	0	1.5
57	14/2 -- 11	12/2 -- 11	0	3.50	1.00	1.25	18.50	27.25	10.50	15.75	15.25	1.25	8	0	91	8	0	2	1	2.5
		19/2 ,	0.25	2.00	1.50	0.25	6.50	14.50	16.00	16.75	21.50	2.25	11	0	36	3	0	1	0	1.9
58	18/3 -- 11	14/3 -- 11	0.75	1.50	0.75	1.00	4.25	11.25	15.75	20.00	21.25	1.00	6	0	68	6	0	3	1	1.2
		20/3 ,	0.25	1.25	1.00	0.75	7.25	20.25	15.50	11.50	16.25	1.25	8	0	58	6	0	0	0	3.1
		31/3 ,	0.25	3.25	0.50	2.25	5.50	17.75	13.75	21.50	10.50	0.50	13	0	111	11	0	3	1	2.1
		28/1 ,	0	1.00	0.25	0.50	6.25	16.00	17.25	13.50	27.50	1.50	10	0	59	6	1	2	1	0.5
59	22/3 -- 11	21/3 -- 11	0.25	2.75	1.00	1.50	7.25	18.00	18.50	11.75	18.25	1.25	15	0	66	2	0	2	0	1.6
		24/3 ,	0	2.00	0.75	2.00	12.50	18.25	20.25	12.75	14.25	0.75	26	0	99	10	3	2	2	1.8
		1/4 ,	0	3.25	0.75	0.25	3.00	18.00	25.50	20.00	13.25	1.50	13	0	76	6	1	0	2	1.3
		19/5 ,	0	1.50	1.00	2.00	8.00	19.75	16.75	18.50	29.75	11.00	11	1	58	12	1	1	2	0.1
60	27/3 -- 11	26/3 -- 11	0	1.75	0.25	0.75	7.00	17.50	12.25	7.25	22.00	1.25	6	0	32	5	0	1	0	0.9
		28/3 ,	0.25	1.25	0.25	0.25	6.50	16.75	16.25	7.00	23.75	3.00	13	0	81	9	1	2	0	1.8
		9/4 ,	0	1.00	0.50	1.00	11.75	17.75	8.00	25.25	25.75	1.50	7	0	18	6	0	1	0	0.7
		23/5 ,	0	2.25	1.00	1.25	6.50	19.00	19.00	11.50	22.25	1.50	9	1	19	9	0	1	1	0.1
61	28/3 -- 11	28/3 -- 11	0.75	1.25	1.00	0.25	1.50	7.25	6.00	5.75	12.00	28.25	7	0	22	1	0	0	0	0.1
		31/3 ,	0.25	2.25	3.00	1.50	5.25	11.00	9.50	6.50	19.75	38.00	5	0	91	7	1	3	0	0
62	3/1 -- 11	2/1 -- 11	0	1.25	0.25	2.00	5.00	12.75	20.75	22.00	28.00	1.00	3	0	34	5	0	2	0	2.4
		1/4 ,	0.25	3.50	1.50	0.75	5.00	16.25	28.75	13.50	15.75	11.75	1	0	39	3	0	1	0	0
		15/4 ,	0.75	4.50	1.75	1.25	6.25	18.25	19.50	10.75	15.75	17.50	7	0	116	12	2	3	5	8.6
		6/6 ,	0	6.75	5.50	0.75	6.00	13.75	16.00	13.50	11.25	1.00	2	0	31	6	0	1	0	1.0

Tabelle 24 (Forts.).  
Die Sternalpunkte der Tonsillitis chronica-Fälle.

Nr.	Datum der Tonsillektomie	Datum	%										Zahl auf 400 L.						Retikulez.	
			Bas. Myeloz. & Leukoz.	Eos. Myeloz.	Eos. Leukoz.	Myelobl.	Neutr. Promyeloz.	Neutr. Myeloz.	Neutr. Metamyeloz.	Neutr. stabk. Leukoz.	Neutr. segm. Leukoz.	Lymphoz.	Monoz.	Retikulum- & Plasmaz.	Megakar.	Nomobl.	Makrobl.	Proerythrobl.	Rote Mitosen	Weisse Mitosen
63	5/4—11	5/4—11 7/4 » 18/4 » 13/3—12	0 0.75 0 0	1.25 2.00 3.25 0.50	0.25 0.25 0.50 0	0.50 0.75 0 0	4.25 4.50 4.25 5.25	14.25 15.00 21.75 19.00	17.75 15.75 20.00 18.00	17.25 17.50 18.75 12.50	25.25 28.00 17.25 19.25	17.50 19.50 12.75 23.75	1.75 1.00 1.50 1.00	7 9 12 5	0 0 0 0	65 76 39 51	5 3 3 3	0 2 1 0	2 2 0 0	1 0 0 0
64	9/4—11	8/4—11 10/4 » 16/6 »	0 0 0	6.25 6.75 2.25	0.25 1.50 0	0.75 2.50 1.25	4.25 9.50 7.50	12.25 19.75 22.00	18.75 16.50 34.75	17.75 10.00 16.00	18.25 13.00 8.50	20.00 17.25 7.50	1.50 3.25 0.25	5 6 3	0 0 0	46 146 54	5 23 8	0 0 1	1 4 2	0 1 0
65	19/4—11	19/4—11 21/4 » 26/5 »	0.25 0.25 0.25	1.75 1.25 2.75	0 1.25 1.25	1.25 1.25 1.25	5.00 4.25 4.00	18.25 20.75 12.25	20.75 17.75 17.25	13.00 12.00 10.00	20.50 16.50 27.25	18.00 21.75 21.50	1.25 3.00 2.00	10 8 0	1 0 0	85 76 36	5 4 0	1 1 0	1 1 0	0 1 0
66	28/4—11	27/4—11 9/5 »	0 0.25	1.75 3.50	1.00 1.25	0.75 2.00	10.50 9.25	14.75 15.25	23.50 19.00	12.50 17.50	18.50 20.25	15.75 10.00	1.00 1.75	0 8	0 0	53 80	3 5	0 0	1 1	0 0
67	3/5—11	26/4—11 5/5 »	0.25 0.25	0.75 2.00	1.00 1.25	1.00 1.75	6.00 7.00	17.75 20.50	18.00 17.25	10.25 9.75	28.25 17.00	14.75 22.25	2.00 1.00	5 9	0 0	77 57	5 2	0 0	3 1	0 0
68	8/5—11	7/5—11 9/5 » 16/3—12	0 0 0	2.25 1.50 2.25	0.75 2.25 0.75	2.25 1.00 0.75	5.25 5.50 4.50	13.50 14.25 15.25	15.50 8.50 13.50	13.50 6.00 10.75	22.50 27.50 25.00	22.00 31.00 25.75	2.50 2.50 1.50	11 5 8	0 0 0	54 51 47	4 4 10	1 4 0	2 0 2	0 0 0

69	13/5—11	12/5—11 15/5 » 16/3—12	0 0 0	2.75 1.75 0 3.50 0.75	0.75 1.25 0.50	10.00 5.25 4.75	23.25 27.00 19.25	21.25 20.00 14.25	16.25 6.50 10.00	12.00 19.50 29.25	10.75 16.75 18.25	1.75 2.00 1.50	8 8 7	0 1 0	62 47 68	1 1 4	0 0 0	1 0 1	1 1 0	0.6 0.6 1.0
70	16/5—11	15/5—11 17/5 » 16/6 » 12/3—12	0.25 0.25 0 0	1.50 0.75 1.00 0.75 2.25 0.50 1.50 0.50	1.00 1.25 1.25 1.25	5.75 7.50 4.25 11.50	13.75 19.75 13.50 22.25	18.00 18.25 15.00 23.50	11.50 14.00 14.50 10.75	25.75 18.75 23.00 11.00	18.75 16.00 25.50 15.75	3.00 2.50 0.25 2.00	11 15 4 6	0 0 0 0	86 63 46 94	5 3 16 32	0 1 0 1	1 1 1 4	0 0 0 2	0.5 1.5 1.2 1.2
71	17/5—11	16/5—11 21/5 » 12/6 »	0 0 0	2.25 0.25 1.00 0.50 1.75 0.50	0.50 1.0 1.50	7.00 19.00 8.00	22.25 20.25 19.00	19.75 19.50 17.25	15.25 13.00 11.75	13.25 10.00 15.75	17.50 14.25 22.75	2.00 1.50 1.75	9 18 11	0 0 0	51 43 33	15 6 11	1 2 0	2 2 1	1 0 0	0.7 1.1 0.8
72	23/5—11	21/5—11 24/5 » 5/6 » 19/3—12	0.25 0 0 0	2.00 1.00 2.25 0.50 2.00 0.50 2.75 0.75	1.00 1.75 1.50 0.50	10.00 12.00 8.50 3.00	19.00 21.00 19.00 15.25	17.75 20.00 22.00 21.00	14.50 10.00 12.50 16.00	10.50 9.00 11.75 17.75	22.75 19.75 18.75 22.00	1.25 0.75 0.50 1.00	19 17 11 10	1 0 0 1	68 61 105 75	13 14 21 7	1 1 1 0	2 1 2 1	0 1 2 0	4.6 6.5 1.4 0.8
73	24/5—11	23/5—11 26/5 » 4/6 »	0 0 0	3.00 0.50 1.00 0.25 3.00 0.25	1.00 0.25 1.50	14.00 9.50 11.00	15.75 14.00 15.25	18.25 19.00 21.75	14.00 13.75 15.75	8.00 21.75 10.75	21.00 21.75 19.75	1.50 2.50 1.00	13 12 7	2 1 0	92 71 90	9 16 9	1 1 1	2 0 1	0 2 0	0.5 0.8 0.5
74	28/5—11	27/5—11 30/5 » 18/3—12	0.25 0 0	2.00 0.50 2.00 0.75 3.00 0.50	1.25 1.75 0.25	8.25 6.25 3.25	18.50 17.50 14.75	20.00 23.50 17.00	15.50 17.50 15.00	11.75 10.00 20.25	21.25 19.25 27.25	0.75 1.00 1.25	1 6 1	0 0 1	37 50 64	4 9 5	0 2 0	0 4 0	0 1 0	1.2 2.8 1.2
75	5/6—11	5/6—11 6/6 »	0 0	1.25 0.25 2.50 0	1.50 1.50	10.75 14.50	22.25 20.25	18.00 18.75	12.00 13.00	10.75 13.00	22.50 15.00	0.75 1.50	6 15	0 0	70 74	7 18	1 1	0 1	0 1	1.2 2.4
76	11/6—11	10/6—11 13/6 » 17/3—12	0 0.25 0	2.00 1.00 2.25 0.25 3.25 1.50	0.25 2.00 0.75	9.25 11.50 4.75	14.75 19.75 13.50	18.50 15.75 18.50	13.00 13.25 12.00	12.50 13.00 20.00	28.50 21.25 21.00	0.25 0.75 1.75	6 7 5	0 0 0	99 52 71	9 6 3	0 0 1	1 0 2	0 0 0	0.9 1.8 0.9
77	16/6—11	14/6—11 18/6 » 13/3—12	0 0 0	2.25 1.00 3.00 0.75 1.75 0.50	1.00 0.50 0.50	4.50 9.75 5.50	11.75 12.50 9.50	9.75 13.25 12.75	11.00 10.00 10.25	27.50 22.50 37.50	30.75 26.00 20.00	0.25 1.75 1.75	12 12 9	1 1 1	34 34 62	6 2 7	0 1 1	0 0 0	1 1 1	0.8 0.8 0.8



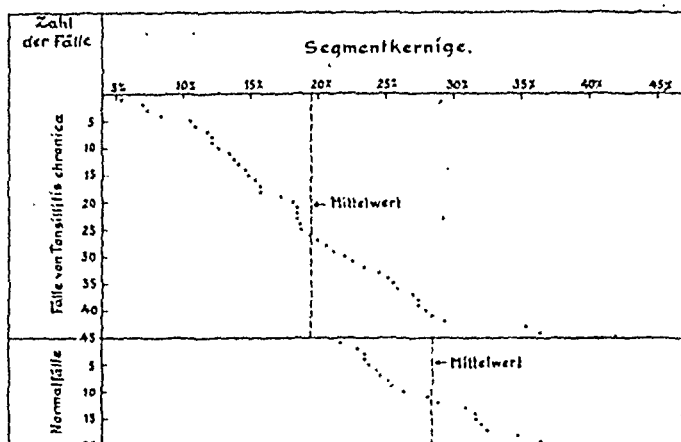


Abb. 26. Werte der Segmentkernigen vor der Tonsillektomie.

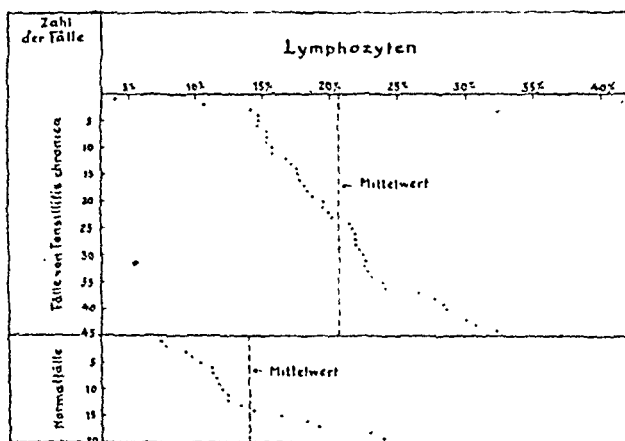


Abb. 27. Lymphozytenwerte vor der Tonsillektomie.

zelen, ausnahmsweise hohen Werten, sondern bei den Patienten kamen allgemein mehr Lymphozyten als in meinen Normalfällen vor. Das erhellt aus Abbildung 27, in der die Variationsgrenzen der Lymphozyten wiedergegeben sind. Die Mittelwerte der Monozyten sind praktisch die gleichen. Auch bezüglich der Plasma- und Retikulumzellen sowie der Megakaryozyten ist kein bemerkenswerter Unterschied zu konstatieren.

Prototypen der Erythrozyten waren bei den Patienten etwas mehr als in den Normalfällen zu finden. Die Differenz war für die

Makroblasten ausgeprägter als für die Normoblasten. Die Zahl der Mitosen war etwas herabgesetzt, die der Retikulozyten stand auf derselben Höhe wie in den Normalfällen.

In Tabelle 28 sind die Sternalpunktate auf Grund des Vorkommens und der Art der bei den Patienten angetroffenen Sekundärsymptome in verschiedene Gruppen eingeteilt. Wie man daraus ersieht, sind zwischen den zu den verschiedenen Gruppen gehörenden Punktaten keine beträchtlichen Unterschiede festzustellen. Prototypen der Neutrophilen kamen am zahlreichsten und Lymphozyten am spärlichsten bei Patienten vor, die keine Sekundärbeschwerden hatten. Fertige Leukozyten fanden sich andererseits am meisten in der Gruppe, in der Begleitkrankheiten auftraten. Ich habe auch geprüft, ob die Punktate je nachdem, wie schwer die Tonsillenveränderungen bei den Patienten waren, Unterschiede aufweisen. Dabei stellte sich heraus, dass die in den Punktaten nachweisbaren Veränderungen nicht der Stärke der in den Tonsillen auftretenden entzündlichen Veränderungen parallel verlaufen. Auch in den fünf Fällen, in denen ausser der Tonsillitis deutliche Zahninfektionen vorlagen, wichen die Sternalpunktate nicht von den anderen ab. Bei diesen Patienten fanden sich fertige Neutrophile durchschnittlich 22 %, Lymphozyten 19 % und myeloische Prototypen zusammen 52 %.

In seiner Untersuchung über den Einfluss der Bothriocephalusinfektion auf die Hämatopoese hat TÖTTERMAN bei einigen seiner Fälle im Sternalpunktat eine leichte Vermehrung der Lymphozyten und eine Linksverschiebung der Neutrophilen sowie hinsichtlich der Erythrozyten oft herabgesetzte Normoblastenwerte festgestellt. In meinem eigenen Material wurde bei 6 Patienten weiter eine Bothriocephalusinfektion gefunden. Die Abweichungen der myeloischen Zellen von der Norm vertraten bei diesen Patienten dieselbe Stufe wie bei den anderen (Neutrophile im Mittel 20 % und Lymphozyten 22 %). Die Zahl der Erythroblasten war nicht erniedrigt (zusammen 82 je 400).

In den ersten Tagen nach der Tonsillektomie.

Wenn man untersuchen will, ob die Tonsillektomie Reaktionen im Sternalmark hervorruft, wäre es am besten, die Patienten täglich zu punktieren. Obwohl die Punktion dem Patienten

Tabelle 28.

Variationsgrenzen und Mittelwerte der Sternalpunktrate vor der Tonsillektomie.  
Die Patienten in verschiedene Gruppen geteilt.

		Ohne Neben- symptome 6 Fälle	Subjektive Neben- symptome 25 Fälle	Objektiv konstatierte Neben- krankheiten 14 Fälle	Normalwerte
Bas. Myeloz. & Leukoz.	%	0—0.25 0.08	0—0.75 0.45	0—0.75 0.15	0—1.0 0.3
Eos. Myeloz.		0.75—3.50 2.21	1.25—6.25 2.59	0.75—8.50 2.0	0.25—8.75 3.08
Eos. Leukoz.		0.25—1.0 0.83	0—3.75 0.93	0—4.0 1.3	0—3.0 1.0
Myelobl.		0.5—1.50 1.04	0.25—3.0 1.39	0.25—4.25 1.6	0.50—3.0 1.23
Neutr. Promyeloz.		5.75—10.50 7.83	1.25—14.0 6.4	1.50—7.0 5.13	2.25—9.25 4.75
Neutr. Myeloz.		14.75—23.25 19.08	7.0—22.25 14.85	7.25—21.0 12.64	8.50—21.50 14.76
Neutr. Metamyeloz.		18.0—27.25 21.38	5.0—28.0 18.07	6.0—25.0 19.14	10.25—24.50 18.44
Neutr. stabk. Leukoz.		10.25—16.25 12.75	7.25—20.0 12.84	5.75—22.0 14.16	8.25—19.0 12.65
Neutr. segm. Leukoz.		12.0—28.25 17.67	6.25—32.25 18.44	14.0—42.0 22.09	21.75—42.75 28.5
Lymphoz.		10.75—19.50 15.58	14.0—37.0 22.63	4.0—30.75 19.6	7.50—25.0 14.06
Monez.		1.25—2.0 1.38	0—4.0 1.25	0.25—3.0 1.50	0.25—3.0 1.26
Retikulum- & Plasmaz.	Zahl auf 400 L.	0—15 7.5	1—25 8.9	2—17 8.2	3—16 8.0
Megakar.		0—0 0	0—3 0.37	0—1 0.4	0—1 0.2
Normobl.		51—91 66.67	11—143 77.72	22—120 64.14	23—111 61.6
Makrobl.		1—15 5.67	1—33 9.24	1—10 5.0	0—10 3.8
Proerythrobl.		0—1 0.17	0—12 0.83	0—3 0.2	0—2 0.35
Rote Mitosen		1—3 1.83	0—4 1.35	0—4 1.0	0—4 1.55
Weisse Mitosen		0—1 0.5	0—5 1.01	0—2 0.61	0—3 0.5
Retikuloz.	%	0.4—2.5 1.3	0.2—4.6 1.6	0.5—3.6 1.8	0.3—2.4 1.2



Tabelle 29.  
Variationsgrenzen und Mittelwerte der Sternalpunkte in den ersten Tagen nach der Tonsillektomie.

Variationsgrenzen und Mittelwerte der Sternalpunkte in den ersten Tagen nach der Tonsillektomie.							
	1—2 T. nach der Tonsillektomie. 35 Fälle		3—8 T. nach der Tonsillektomie. 11 Fälle		9—26 T. nach der Tonsillektomie. 18 Fälle		Normalwerte
	Werte vor der Operation	Werte nach der Operation	Werte vor der Operation	Werte nach der Operation	Werte vor der Operation	Werte nach der Operation	
Bas. Myeloz. & Leukoz.	0—0.75 0.25	0—0.75 0.15	0—0.75 0.11	0—0.25 0.15	0—0.75 0.25	0—0.75 0.2	0—1.0 0.3
Eos. Myeloz.	0.75—8.50 2.65	0.50—6.75 2.35	0.75—5.0 2.75	1.0—3.25 1.98	1.25—8.50 3.0	1.0—6.0 3.15	0.25—8.75 3.08
Eos. Leukoz.	0—3.75 0.95	0—3.25 0.9	0—1.0 1.1	0.25—3.75 1.55	0.25—2.75 1.15	0.25—1.75 1.5	0—3.0 1.0
Myelobl.	0.25—4.25 1.48	0—2.50 1.4	0.25—2.75 1.4	0.25—2.25 1.2	0.50—1.25 1.65	0—3.75 1.4	0.50—3.0 1.23
Neutr. Promyelo.	1.25—14.0 6.55	1.75—14.50 6.83	1.50—7.25 4.6	1.0—6.75 4.35	1.25—14.0 7.1	1.75—12.50 5.5	2.25—9.25 4.75
Neutr. Myeloz.	7.0—23.25 15.05	4.75—27.0 15.7	7.25—21.0 14.05	7.75—18.25 14.23	7.25—21.75 14.6	8.25—21.75 14.95	8.50—21.50 14.76
Neutr. Metamyeloz.	5.0—28.0 18.55	4.75—28.75 17.15	6.0—27.25 21.98	8.0—24.50 19.75	11.75—28.0 18.95	10.0—25.50 19.1	10.25—21.50 18.41
Neutr. stabk. Leukoz.	6.75—22.0 13.53	3.50—17.50 11.7	5.75—21.75 12.15	5.75—18.0 12.65	7.25—22.0 14.68	8.0—20.0 13.8	8.25—19.0 12.65

Neutr. segm. Leukoz.	6.25—36.75 18.95	9.0—46.50 21.3	13.25—42.0 18.8	13.75—24.50 20.05	6.25—29.0 17.57	6.75—41.75 20.25	21.75—42.75 28.5
Lymphoz.	4.0—37.0 20.55	14.25—38.50 21.1	15.25—28.25 21.4	15.0—38.0 22.85	4.0—29.0 19.9	10.0—35.0 19.55	7.50—25.0 14.06
Monoz.	0—4.0 1.3	0—3.50 1.48	0—3.0 1.15	0—3.25 1.35	0.25—2.75 1.2	0—3.0 1.1	0.25—3.0 1.26
Retikulum- & Plasmaz.	1—19 8.5	1—26 9.15	2—25 11.25	3—20 10.52	0—25 9.4	3—17 8.5	3—16 8.0
Megakar.	0—2 0.4	0—1 0.17	0—1 0.2	0—0 0	0—1 0.4	0—2 0.37	0—1 0.2
Nornobl.	11—138 68.55	14—146 58.7	22—143 69.5	26—108 56.55	34—143 71.8	13—139 72	23—111 61.6
Makrobl.	1—33 7.45	0—23 6.3	1—24 6.1	0—16 4.15	2—33 10.45	2—22 8.25	0—10 3.8
Proerythrobl.	0—4 0.48	0—3 0.58	0—12 1.3	0—7 0.63	0—12 0.75	0—3 0.55	0—2 0.35
Rote Mitosen	0—4 1.3	0—5 1.2	0—4 1.2	0—4 0.95	0—4 1.65	0—3 1.3	0—4 1.55
Weisse Mitosen	0—4 0.77	0—2 0.57	0—5 1.05	0—1 0.28	0—5 1.15	0—5 0.63	0—3 0.5
Retikuloz.	0.2—1.6 1.6	0.6—4.5 2.0	0.7—3.6 2.2	0.4—2.1 1.4	0.2—4.6 1.7	0.4—8.6 1.8	0.3—2.4 1.2

selten direkte Schmerzen verursacht, betrachten die meisten sie doch als unangenehm und unterwerfen sich nicht gern wiederholt dieser Massnahme. Aus diesem Grunde habe ich es unterlassen, die Punktion alltglich durchzufhren. Ich habe versucht, den Einfluss der Tonsillektomie so aufzuklren, dass ich die Patienten verschiedenen lange Zeit nach der Operation punktierte. So wurden im Laufe von 3 Wochen nach der Tonsillektomie insgesamt 64 Sternalpunktionen gemacht. Beim Vergleich der in den ersten Tagen ausgefhrten verschiedenen Punktionen habe ich gefunden, dass kein wesentlicher Unterschied festzustellen war. Eine allgemeine Vorstellung von den Ergebnissen dieser Punktionen gibt Tabelle 29, in der die Punktionen in drei Gruppen zusammengefasst sind je nachdem, wie lange nach der Operation die Punktionen ausgefhrt wurden. Zum Vergleich sind auch die Mittelwerte fr die vor der Operation entnommenen Punktate bei den zu den einzelnen Gruppen gehrenden Patienten berechnet. Wie aus der Tabelle hervorgeht, ist in den Zellen der myeloischen Gruppe im Laufe dreier Wochen kein deutlicher Unterschied gegenber den vor der Operation gefundenen Werten zu bemerken. Dagegen sind die Mittelwerte der Prototypen der Erythrozyten in den zwei ersten Gruppen kleiner als die entsprechenden Zahlen vor der Operation. Die geringere Zahl der Erythroblasten kann eine leichte myeloische Hyperplasie widerspiegeln, die jedoch so unbedeutend ist, dass sie bei der Beurteilung der Zellfrequenz in den Prparaten nicht mit Sicherheit festzustellen war. In der dritten Gruppe ist die erwhnte Verschiedenheit nicht nachzuweisen.

Im Zusammenhang mit der Tonsillektomie erkrankten 2 Patienten an einer Bronchopneumonie (57 und 71), 1 bekam eine fieberhafte Schnupfeninfektion (72), und bei 1 trat eine deutliche, vorbergehende Exazerbation der Gelenkbeschwerden auf (49). In Fall 71 war gleichzeitig eine betrchtliche Vermehrung der Promyelozyten und bei dem Patienten 57 eine Zunahme der Stab- und Segmentkernigen zu konstatieren. Bei beiden zeigte sich ausserdem auch eine Erhhung des Erythroblasten-Leukozytenindex. In den Fllen 49 und 72 war keine deutliche Reaktion zu finden.

In 5 Fllen konnte ich feststellen, dass die Zahnextraktion keine unmittelbare Reaktion im Knochenmark bewirkt hatte.

Tabelle 30.

Variationsgrenzen und Mittelwerte der Sternalpunkttate bei der Spät-Nachuntersuchung.

		Bei der Spät-Nachuntersuchung symptomlos. 22 Fälle		Bei der Spät-Nachuntersuchung Symptome. 8 Fälle		Normalwerte
		Werte vor der Operation	Werte bei der Nachuntersuchung	Werte vor der Operation	Werte bei der Nachuntersuchung	
Bas. Myeloz. & Leukoz.	%	0—0.75 0.28	0—0.75 0.11	0—0.50 0.19	0—0.50 0.06	0—1.0 0.3
Eos. Myeloz.		1.25—8.50 2.72	0.50—7.0 2.91	1.50—6.25 2.81	0.75—3.75 1.97	0.25—8.75 3.08
Eos. Leukoz.		0—2.75 0.95	0—5.50 1.05	0.25—1.50 0.91	0—1.75 0.78	0—3.0 1.0
Myelobl.		0.25—2.75 1.47	0—3.75 0.12	0.50—2.50 1.34	0.50—2.0 1.03	0.5—3.0 1.23
Neutr. Promyeloz.		1.25—10.50 6.03	1.75—8.0 4.75	1.75—10.0 5.28	3.0—11.50 6.0	2.25—9.25 4.75
Neutr. Myeloz.		7.25—23.25 14.39	6.75—19.75 14.57	8.50—21.0 14.16	9.50—22.25 15.38	8.50—21.50 14.76
Neutr. Metamyeloz.		11.75—28.0 19.45	10.25—24.50 17.53	9.75—22.50 17.16	8.75—34.75 19.19	10.25—24.50 18.44
Neutr. stabk. Leukoz.		7.25—22.0 14.11	9.25—18.50 12.24	8.25—17.75 12.91	10.25—16.0 13.63	8.25—19.0 12.65
Neutr. segm. Leukoz.		8.25—29.50 19.24	14.25—45.75 24.18	10.50—37.50 21.75	8.50—37.50 20.31	21.75—42.75 28.5
Lymphoz.		4.0—30.0 19.94	11.0—27.25 20.18	17.75—30.75 21.72	7.50—26.50 20.09	7.50—25.0 14.06
Monoz.		0—2.75 1.23	0—2.50 1.32	0.25—3.0 1.75	0.25—3.0 1.63	0.25—3.0 1.26
Retikulum- & Plasmaz.	Zahl auf 400 L	1—25 8.5	1—14 6.5	2—19 8.38	3—14 8.62	3—16 8.0
Megakar.		0—1 0.36	0—1 0.23	0—1 0.5	0—1 0.25	0—1 0.2
Normobl.		34—143 69.5	29—121 61.91	34—88 63.12	31—94 64.88	23—111 61.6
Makrobl.		1—33 7.9	0—14 5.77	1—13 5.5	3—32 9.62	0—10 3.8
Proerythrobl.		0—12 0.59	0—1 0.23	0—1 0.25	0—1 0.62	0—2 0.35
Rote Mitosen		0—4 1.09	0—2 0.95	0—2 1.12	0—4 1.12	0—4 1.55
Weisse Mitosen		0—5 0.95	0—3 0.5	0—1 0.38	0—2 0.38	0—3 0.5
Retikuloz.	%	0.2—2.9 1.4	0.2—2.5 1.0	0.5—4.6 2.4	0.8—1.5 1.1	0.3—2.4 1.2

### Bei der Spät-Nachuntersuchung.

Wie oben erwähnt, wurde eine Spät-Nachuntersuchung bei 30 Patienten ausgeführt. Bei 4 von diesen war damals noch eine Sekundärkrankheit und bei 4 eine zufällige akute Infektion vorhanden. Bei den übrigen 22 waren die subjektiven und objektiven Sekundärsymptome verschwunden. Nur die letztgenannten können berücksichtigt werden, wenn man die Einwirkung der Tonsillektomie auf das Sternalmark prüft. (Hierzu sind auch die 5 zur Nachuntersuchung gekommenen Fälle gerechnet, die zugleich Zahnbehandlung erhalten hatten.) Im Zellgehalt der Punktrate waren keine Abweichungen von der Norm festzustellen. Vergleicht man die Mittelwerte mit den vor der Operation gefundenen Werten und den Normalwerten in Hinsicht auf die Korrelation der Zellen (Tabelle 30), so ergibt sich, dass die vor der Operation auftretende leichte Linksverschiebung der Neutrophilen bei der Nachuntersuchung verschwunden ist. Die prozentualen Mengen der Prototypen der Neutrophilen stehen den Normalwerten sehr nahe. Die Zahl der Segmentkernigen ist höher als vor der Tonsillektomie, aber fortwährend doch niedriger als in den Normalfällen. In bezug auf die Lymphozyten ist kein Unterschied wahrzunehmen. Ihre prozentuale Menge ist dieselbe wie vor der Operation und mithin eine höhere als in den Normalfällen. Die Werte der Erythrozyten waren etwas niedriger als vor der Operation.

Die Mengen der Retikulozyten und der roten Mitosen sind praktisch bei beiden Untersuchungen die gleichen. Die Zahl der weissen Mitosen war bei der Nachuntersuchung etwas geringer als vor der Tonsillektomie.

Tabelle 30 enthält auch die Mittelwerte der Sternalpunktate derjenigen 8 Patienten, die bei der Nachuntersuchung nicht symptomlos waren. Bei diesen Patienten ist immer noch eine Linksverschiebung der Neutrophilen zu bemerken, und zwar sogar deutlicher ausgeprägt als vor der Tonsillektomie. Die prozentuale Menge der Lymphozyten ist bei ihnen ebenfalls hoch.









Tabelle 31 (Forts.)

Nr.	Datum der Ton- sillekto- mie	Datum	Hb	Erythroz.	Index	Leukoz.	Neutroph.	Meta- myeloz.	Stab- kernige	Segment- kernige	Basoph.	Eosinoph.	Monoz.	Lymphoz.	Throm- boz.	Retikuloz.	SR
52	21/11—40	20/11—40	80/86	4460	0.97	6400	49.4	0.2	9.6	39.6	0	1.4	9.0	40.2	197100	0.5	24
		23/11 »	80/86	4430	0.97	6700	46.0	0	9.8	36.2	1.2	1.8	8.2	42.8	200100	0.5	21
		25/11 »	79/85	4450	0.96	7430	49.6	0	9.8	39.8	1.2	2.6	6.0	40.6	242000	0.3	24
		29/11 »	78/84	4220	1.00	7300											16
		12/2 —41	79/85	4072	1.04	4600	64.8	0	2.4	62.4	0.2	1.6	9.0	24.4	236200	1.0	5
53	12/12—40	12/12—40	74/80	4300	0.93	5560	51.4	0.2	7.6	43.6	1.0	4.8	11.0	31.8	258000	1.7	30
		16/12 »	60/65	3689	0.89	5020	53.4	0.2	4.4	48.8	0.6	3.2	9.0	33.8	199000	1.8	35
		31/1 —41	78/84	4610	0.91	5300	58.0		6.0	52.0	1.8	7.0	10.6	28.8	198000		10
		20/3 »	80/86	4540	0.94	4850	41.0	0	1.4	39.6	1.4	2.2	6.4	49.0	216000	0.5	5
		8/1 —41	86/93	4760	0.97	8660	58.2	0.2	3.4	54.6	0.2	2.4	10.0	29.2	238000	0.8	12
54	9/1 —41	11/1 »	85/92	4650	0.99	10100	71.0	0	4.2	66.8	0.2	1.6	6.0	21.2	198000	0.9	12
		15/1 »	80/86	4720	0.98	7800	56.8	0	6.0	50.8	0.6	3.4	10.2	29.0	285000	0.6	12
		26/3 »	80/86	4150	1.03	5400	39.0	0	5.0	34.0	1.0	4.6	10.6	44.8	203000		6
		5/2 —41	81/87	4740	0.92	6500	58.4	0	4.6	53.8	0	5.2	8.8	27.6	237000	1.5	7
		8/2 »	80/86	4660	0.92	6600	61.6	0	7.0	54.6	0.6	4.6	6.4	26.8	233000	1.9	8
55	7/2 —41	11/2 »	79/85	4700	0.90	7060	56.8	0	8.8	48.0	0	5.6	7.4	30.2	198000	1.4	17
		18/2 »	80/86	4540	0.96	6100	59.2	0	6.0	53.2	0.2	5.4	6.6	28.6	181600	0.8	13
		20/2 »	80/86	4400	0.98	5600	57.4	0	6.2	51.2	0.2	6.4	6.6	29.4	211200	0.9	11
		3/3 »	80/86	4150	1.05	5100	62.0	0	6.4	55.6	1.2	7.2	6.2	23.2	198500	0.9	14
		10/5 »	90/97	4950	0.90	4550	62.0	0	6.4	55.6	0	2.6	1.8	33.6	217400	0.1	4
56	7/2 —41	16/3 —42	84/91	5300	0.86	4900	77.8	0	12.0	65.8	0	0.8	2.0	19.4	230000	0.3	2
		5/2 —41	65/70	4460	0.78	6600	64.4	0.2	4.2	60.0	0.6	3.2	5.4	26.4	267000	0.9	31
		8/2 »	65/70	4420	0.79	6700	69.6	0	4.0	65.6	0.2	1.2	3.8	25.2	208000	0.8	30
		11/2 »	65/70	4410	0.79	6100	63.4	0	3.4	60.0	1.4	2.2	4.8	28.2	198000	0.9	42
		17/2 »	66/71	4480	0.81	6800	58.4	0	4.0	54.4	1.6	3.2	4.2	32.6	224600	0.5	15
57	14/2 —41	16/4 »	64/69	4230	0.81	9100	77.2	0	6.2	71.0	0.2	2.4	6.8	13.4	198000		20
		12/2 —41	91/98	5250	0.93	7930	52.8	0	5.2	47.6	0.2	2.0	6.6	38.4	272000	0.3	2
		15/2 »	91/98	5100	0.96	15900	83.0	0	20.6	62.4	0	0.2	5.6	11.0	244800	0.2	21
		19/2 »	89/96	4900	0.98	7560	50.0	0	5.4	44.6	0.2	2.2	9.2	38.2	235000	0.4	65



Tabelle 31 (Forts.)

Nr.	Datum der Ton- sillekto- mie	Datum	Hb	Erythroz.	Index	Leukoz.	Neutroph.	Meta- myeloz.	Stab- ferrnige	Segment- kernige	Basoph.	Eosinoph.	Monoz.	Lymphoz.	Throm- boz.	Retikuloz.	SR
69	13/5—41	12/5—41 16/3—42	75/81 65/70	4310 4140	0.94 0.85	7800 5000	79.6 61.0	0 0	11.2 6.8	68.4 54.2	0 0	1.4 0.6	3.4 5.2	15.6 33.2	189600 207000	0.3 0.3	13 8
70	16/5—41	15/5—41 17/5 » 16/6 » 12/3—42	78/84 78/84 78/84 76/82	4220 4080 3620 3820	0.86 1.05 1.11 1.07	4900 8200 3800 7900	42.4 59.8 67.8	0 0.2 0	6.2 5.4 11.4	36.2 54.2 56.4	0.8 2.6 0.2	6.2 2.2 0.6	11.2 10.2 13.0	39.4 25.2 18.4	265800 275400 198000	0.5 0.4 0.3 0.3	18 40 9 13
71	17/5—41	17/5—41 23/5 » 26/5 » 12/6 »	82/89 76/82 82/89	4590 4300 3970	0.90 0.95 1.14	9550 12300 8950	66.4 68.8 72.2	0 0 0.4	1.2 12.2 4.6	65.2 56.6 67.2	1.4 0.2 0	1.2 1.4 1.0	5.8 8.4 5.0	25.2 21.2 21.8	216000 95200	0.9 0.5	8 60 10 7
72	23/5—41	23/5—41 26/5 » 5/6 » 19/3—42	79/85 78/84 80/86 70/76	4380 4320 3830 4800	0.99 0.98 1.13 0.80	8600 7200 6000 5200	62.0 57.6 73.4 58.8	0 0 0	5.4 10.2 3.6 17.2	56.6 47.4 69.8 41.6	0.2 0.2 0.6 0	1.4 2.4 0 0.4	6.2 4.2 5.6 5.4	30.2 35.6 20.4 35.4	214600 177400 268000 244000	0.3 0.7 0.4	20 11 5
73	24/5—41	24/5—41 26/5 » 4/6 »	98/106 97/105 86/93	5060 5150 4600	1.02 1.00 1.01	5760 9100 4150	54.6 68.6 49.4	0 0 0	5.4 12.2 2.6	49.2 56.4 46.8	0 0.2 0.2	1.4 1.6 2.4	7.4 4.4 5.6	36.6 25.2 42.4	202400 199000 182000	0.5 0.3 0.2	3 15 7
74	28/5—41	28/5—41 29/5 » 18/3—42	78/84 78/84 75/81	4050 4310 4190	1.05 0.97 0.96	7900 9400 3800	66.2 74.6 63.2	0 0 0	0.8 6.2 4.2	65.4 68.4 59.0	0.6 0.4 0	1.6 0.4 2.8	5.8 6.4 2.0	25.6 18.2 32.0	242100 215500 192000	1.5 1.5 0.6	19 25 7
75	5/6—41	5/6—41 7/6 »	70/76 72/78	4150 3915	0.91 1.00	7100 14000	56.4 73.0	0 0.4	4.4 7.0	52.0 65.6	0.4 0	0 0	3.4 2.0	39.8 24.0	158000 184000	0.4 0.6	6 9
76	11/6—41	10/6—41 12/6 » 17/3—42	78/84 78/84 68/73	3735 3995 4200	1.14 1.08 0.83	6000 6650 5300	68.2 68.2 64.8	0.2 0	5.8 10.0 3.4	62.2 58.0 61.4	0.4 0.4 0.2	1.0 0.4 1.4	3.0 3.0 4.6	27.4 27.6 29.0	168000 259600 201000	0.8 0.3 0.3	5 9 6
77	16/6—41	14/6—41 17/6 » 13/3—42	70/76 71/77 77/83	3695 3920 4820	1.06 0.99 0.86	4950 7250 5000	58.8 75.6 73.4	0 0.2 0	2.8 4.4 6.6	56.0 71.0 66.8	0.2 0.2 0	0.4 0.8 0.6	3.2 4.8 2.8	37.4 18.6 23.2	251200 141100 241000	1.3 0.5 0.2	9 15 12

Tabelle 32.

Variationsgrenzen und Mittelwerte der Blutbilder vor der  
Tonsillektomie für alle Fälle.

	Tonsillitis chr.	Normalwerte
Hb	63.68—100/108 77.83	75/81—101/109 86.93
Erythroz.	3695—5600 4556	3968—5960 4621
Index	0.68—1.14 0.92	0.74—1.06 0.99
Leukoz.	3850—9800 6574	3800—8300 5975
Neutroph.	42.4—79.6 57.2	47.0—72.0 60.4
Metamyeloz.	0—0.4 0.06	0—0 0
Stabkernige	1.0—11.2 4.9	0.4—4.6 2.6
Segmentkernige	36.2—68.4 52.2	45.0—68.6 57.8
Basoph.	0—2.0 0.4	0—1.6 0.6
Eosinoph.	0—9.0 2.7	0.2—3.6 2.0
Monozyt.	2.4—14.2 7.0	1.0—10.8 4.9
Lymphozyt.	15.6—46.2 32.6	21.0—43.4 32.3
Thromboz.	136500—380400 227763	193300—347800 267980
Retikuloz.	0—2.6 0.6	0.2—1.0 0.4
SR	2—47 12.3	1—10 5.9

der »Kernverschiebungsindex« (das Verhältnis der Prototypen zu den Segmentkernigen)  $\frac{1}{11}$ . SCHILLING gibt als Normalwert  $\frac{1}{16}$  an, und in meinen Normalfällen betrug dieser  $\frac{1}{22}$ . Bei den Fällen mit Linksverschiebung zeigten die Krankenberichte nicht ein häufigeres Vorkommen akuter Infektionen zur Zeit der Untersuchung oder kurz vorher als bei den anderen Patienten. In bezug auf die Eosinophilen und Basophilen war nichts von der Norm Abweichendes festzustellen. Der Mittelwert der Monozyten war etwas höher als in meinen Normalfällen. Mehr als 10 % Monozyten hatten 6 Patienten. Die Werte der Lymphozyten wichen nicht von meinen Normalwerten ab. Ihre prozentuale Menge stimmte durchschnittlich mit derjenigen in den Normalfällen überein, und die absoluten Werte fielen in den Bereich der normalen Variationsbreite. — Die Thrombozyten und Retikulozyten liessen keine Abweichungen von der Norm erkennen.

Tabelle 33 gibt weitere Aufschlüsse über die Frage, ob in den Blutbildern Verschiedenheiten auftreten je nachdem, ob die Patienten Sekundärsymptome haben oder nicht. Wie in den Sternalpunktaten sind auch in den Blutbildern keine erheblichen Abweichungen der Mittelwerte der verschiedenen Gruppen zu konstatieren. Bei 6 Fällen ohne Nebensymptome waren das durchschnittliche Hämoglobinprozent sowie die Leukozytenzahl etwas höher als in den anderen Gruppen. Eine Linksverschiebung der Neutrophilen zeigte sich am deutlichsten bei Patienten, die objektiv nachweisbare Sekundärkrankheiten hatten. Zu dieser Gruppe gehören auch 4 von den 5 Fällen, bei denen ausser der Tonsillitis eine Zahninfektion vorlag. Der absolute Lymphozytenwert war in allen drei Gruppen der gleiche.

Die Senkungsreaktion der Patienten betrug durchschnittlich 12 mm/St, entsprechend dem Wert 6 mm in den Normalfällen. Bei der Betrachtung der verschiedenen Gruppen in Tabelle 33 sieht man, dass der Senkungswert bei den Patienten mit Begleiterkrankungen durchschnittlich doppelt so hoch war wie in den anderen Gruppen, zwischen denen nur ein unwesentlicher Unterschied bestand. Bei genauerer Analyse der einzelnen Werte ergibt sich folgendes. Betrachtet man als obere Grenze des Normalwertes bei den Männern 10 mm und bei den Frauen 12 mm (Seite 23), so findet man am zahlreichsten (in 50 %) erhöhte Werte bei Patienten mit

Tabelle 33.

Variationsgrenzen und Mittelwerte der Blutbilder vor der Tonsillektomie.  
Die Patienten in verschiedene Gruppen geteilt.

	Ohne Neben- symptome 6 Fälle	Subjektive Nebensymptome 25 Fälle	Objektiv konsta- tierte Neben- krankheiten 14 Fälle	Normalwerte
Hb	75/81—91/98 81/87	63/68—98/106 76/82	65/70—100/108 77/83	75/81—101/109 86/93
Erythrozyt.	4310—5250 4723	3735—5120 4513	3695—5600 4563	3968—5960 4621
Index	0.89—0.99 0.93	0.68—1.14 0.92	0.78—1.06 0.92	0.84—1.06 0.99
Leukozyt.	6330—9800 8502	3850—8660 6142	4200—8600 6520	3800—8900 5975
Neutroph.	52.8—79.6 65.7	42.4—71.0 56.1	49.4—64.4 56.3	47.0—72.0 60.4
Metamyeloz.	0—0.4 0.1	0—0.2 0.1	0—0.4 0.1	0—0 0
Stabkernige	1.2—11.2 5.1	1.0—10.6 4.1	2.8—11.0 6.3	0.4—4.6 2.6
Segment- kernige	47.6—68.4 60.5	36.2—65.6 51.5	39.6—60.6 49.9	45.0—68.6 57.8
Basoph.	0—1.4 0.4	0—2.0 0.5	0—1.2 0.5	0—1.6 0.6
Eosinoph.	0.4—4.2 2.1	0—6.6 2.3	0.2—9.0 3.4	0.2—3.6 2.0
Monozyt.	3.4—6.6 5.3	2.4—14.2 7.2	3.2—11.0 7.2	1.0—10.8 4.9
Lymphozyt.	15.6—38.4 26.6	18.4—46.2 34.1	24.6—40.2 32.5	21.0—43.4 32.2
Thrombozyt.	163300—286800 229350	148000—380400 227514	136500—274400 227422	193300—347800 267980
Retikulozyt.	0.3—0.4 0.4	0—2.6 0.6	0.2—1.7 0.8	0.2—1.0 0.4
SR	2—16 9.0	3—47 10.1	2—47 17.6	1—10 5.9

Begleiterkrankungen. In dieser Gruppe war die SR über 30 mm bei 4 Patienten, von denen einer (56) neben der Tonsillitis an Nephritis und 3 (48, 53 und 62) an einer rheumatischen Infektion litten. Bei 7 von 25 Patienten, die ausser der Tonsillitis verschiedenartige subjektive Beschwerden, aber keine objektiv nachweisbaren Krankheiten hatten, lag die SR oberhalb der angeführten Grenzwerte. Unter den 6 sekundärsymptomlosen Fällen der ersten Gruppe wurde bei 2 ein leicht erhöhter Senkungswert angetroffen.

Zwischen der Senkungsreaktion und den Knochenmarksveränderungen war kein Parallelismus nachzuweisen. Das ergibt sich u. a. deutlich bei den Patienten mit Begleiterkrankungen, bei denen das Knochenmarksbild nicht von dem der anderen abwich, aber die SR erhöht war. Auch zwischen der im Blutbild hervortretenden Linksverschiebung und den erhöhten Senkungswerten herrschte keine Übereinstimmung. Linksverschiebung fand sich sowohl bei normaler als auch bei erhöhter SR.

#### In den ersten Tagen nach der Tonsillektomie.

Der Einfluss der Tonsillektomie auf das Blutbild wurde ebenso wie der auf das Sternalpunktat untersucht, indem die Blutbilder in verschiedenen Fällen verschieden lange Zeit nach der Operation verfolgt wurden. Die Ergebnisse finden sich in Tabelle 34, die die Variationsgrenzen und die Mittelwerte aus insgesamt 76 Blutbildern enthält. Einen Tag nach der Tonsillektomie zeigten die Mittelwerte eine Zunahme der Gesamtzahl der Leukozyten, der prozentualen Menge der Neutrophilen und der Linksverschiebung sowie eine relative, aber nicht absolute Lymphopenie, also typische Veränderungen, wie man sie im Anfangsstadium von Infektionen findet. Eine ähnliche Reaktion ist ferner 2 Tage nach der Operation zu bemerken, dagegen nicht mehr in den späteren Blutbildern, wo ihre Mittelwerte im grossen und ganzen von derselben Art wie vor der Operation sind. Die Werte des roten Blutbildes sind nach der Tonsillektomie regelmässig niedriger als bei der ersten Untersuchung. Die Unterschiede sind jedoch gering. Die Zahnsanierung wurde in 5 Fällen zu einer Zeit ausgeführt, als die durch die Ton-

sillektomie verursachte Leukozytose und die Reaktion auf die Linksverschiebung schon aus dem Blutbild verschwunden waren. Bei der Extraktion der Zähne waren keine neuen Veränderungen im Blutbild zu beobachten.

In den Senkungswerten tritt die durch die Operation ausgelöste Reaktion deutlich hervor. Sie sind 1—11 Tage nach der Tonsillektomie höher als vor dem Eingriff. Bei den späteren Untersuchungen ist keine Erhöhung der SR mehr festzustellen. Die grössten Schwankungen wurden in den Werten, wie natürlich, in den Fällen 57 und 71 angetroffen, bei denen sich als postoperative Komplikation eine Pneumonie entwickelte.

#### Bei der Spät-Nachuntersuchung.

Die Variationsgrenzen und Mittelwerte der Blutbilder bei den 30 zur Spät-Nachuntersuchung erschienenen Patienten sind in Tabelle 35 aufgeführt. Bei den 22 symptomlosen Patienten der ersten Gruppe war das durchschnittliche Hämoglobinprozent fortwährend niedrig, ja ein paar Prozent geringer als vor der Tonsillektomie. In 15 Fällen war es nach der ersten Untersuchung herabgesetzt und nur bei 7 Patienten etwas erhöht. Nach der Tonsillektomie zeigte sich also keine Tendenz zum Verschwinden der niedrigen Hämoglobinwerte. Bezüglich des weissen Blutbildes war dagegen ein Abklingen der vor der Tonsillektomie festgestellten Infektionsveränderungen zu beobachten. Eine Linksverschiebung war nicht mehr zu finden. Kein Patient hatte Metamyelozyten, und der Mittelwert der Stabkernigen betrug 3 %. Der Kernverschiebungsindex war  $\frac{1}{16}$ . Auch der frühere ziemlich hohe Monozytenwert war bei der Nachuntersuchung derselbe wie in den Normalfällen.

Der Mittelwert der Senkungsreaktion, der vor der Tonsillektomie mässig erhöht (13) war, war bei der Nachuntersuchung normal (7). Von den einzelnen Werten lagen 2 (Fall 62 SR 13 und Fall 44 SR 12) oberhalb der früher erwähnten normalen oberen Grenze. In Fall 62 wurde die Nachuntersuchung 6 Wochen nach der Operation bei einem Patienten ausgeführt, der an einer akuten rheumatischen Infektion gelitten hatte, weshalb es natürlich ist, dass die Senkungsreaktion, die bei der ersten Untersuchung 47 be-



Tabelle  
Variationsgrenzen und Mittelwerte der Blutbilder

	1 T. nach der Tonsillektomie 29 Fälle.		2 T. nach der Tonsillektomie 11 Fälle		3—4 T. nach der 6
	Werte vor der Operation	Werte nach der Operation	Werte vor der Operation	Werte nach der Operation	Werte vor der Operation
Ilb	63/68—100/108 76/82	61/66—100/108 75/81	70/76—98/106 80/86	55/59—97/105 78/85	65/70—81/87 73/79
Erythro.	3695—5600 4582	3820—5850 4580	4150—5120 4641	3070—5160 4460	4300—4740 4525
Index	0.68—1.08 0.91	0.69—1.08 0.90	0.80—1.02 0.91	0.79—1.0 0.95	0.78—0.99 0.88
Leukoz.	3850—9800 6042	5300—15900 8327	4600—9600 7097	5800—14000 8530	5560—8600 7078
Neutroph.	42.4—71.0 56.4	50.2—83.2 67.7	49.2—66.6 58.1	46.0—88.0 68.3	50.4—64.4 56.7
Metamyeloz.	0—0.4 0.1	0—1.6 0.2	0—0.4 0.1	0—0.6 0.1	0—0.4 0.1
Stabkernige	1.0—11.0 4.7	1.0—20.6 5.4	1.8—10.0 4.5	1.0—12.2 6.3	4.2—7.6 5.2
Segmentker- nige	36.2—65.6 51.1	44.2—77.6 62.4	39.6—62.4 52.6	36.2—75.0 59.5	43.6—60.0 51.3
Basoph.	0—1.2 0.3	0—2.6 0.4	0—2.0 0.5	0—2.0 0.5	0—1.2 0.6
Eosinoph.	0.2—9.0 3.0	0—6.5 1.6	0—4.2 1.9	0—3.4 1.6	1.4—5.2 3.4
Mono.	2.4—12.0 6.7	3.0—10.2 6.7	3.4—14.2 7.7	3.0—13.4 7.1	3.4—11.0 7.6
Lymphoz.	18.4—46.2 34.0	11.0—44.0 24.2	23.2—40.2 31.8	14.4—42.8 24.9	26.4—37.6 31.7
Thromboz.	148000—330000 230141	141100—374300 220109	158000—380400 229909	153500—225400 187909	136500—267000 218350
Retikuloz.	0—2.6 0.7	0.1—1.9 0.7	0.2—0.8 0.4	0.3—0.9 0.5	0.3—1.7 1.0
SR	2—47 13.3	3—55 21.5	3—24 8.5	5—38 14.5	7—38 21.7

34.  
in den ersten Tagen nach der Tonsillektomie.

Tonsillektomie Fälle	5—11 T. nach der Tonsillektomie 20 Fälle		13—26 T. nach der Tonsillekto- mie 6 Fälle		Normalwerte
Werte nach der Operation	Werte vor der Operation	Werte nach der Operation	Werte vor der Operation	Werte nach der Operation	
60/65—79/85 70/76	63/68—98/106 76/82	63/68—91/98 73/79	63/68—82/89 75/81	65/70—80/86 75/81	75/81—101/109 86/93
3690—1700 4344	4040—5250 4657	4260—5100 4598	4130—5040 4641	4000—4965 4373	3968—5960 4621
0.79—0.98 0.87	0.68—1.02 0.89	0.68—1.03 0.88	0.68—1.14 0.83	0.70—1.05 0.93	0.84—1.06 0.99
5020—7200 6505	3850—9600 6422	3900—15900 6495	3850—8600 6458	4100—7900 5708	3800—8900 5975
53.4—63.4 57.7	45.6—66.6 55.1	39.0—83.0 55.4	48.0—63.8 56.2	45.2—71.6 58.9	47.0—72.0 60.4
0—0.2 0	0—0.2 0.1	0—1.6 0	0—0.2 0	0—0 0	0—0 0
1.0—10.2 5.6	1.0—11.0 4.7	1.5—20.6 4.9	3.4—7.4 5.2	0.5—17.4 5.9	0.4—4.6 2.6
47.4—60.0 52.7	38.4—63.0 50.0	34.0—64.5 51.4	42.0—56.2 51.0	43.0—68.0 53.0	45.0—68.6 57.8
0—1.4 0.6	0—2.0 0.3	0—1.6 0.5	0—1.2 0.4	0—1.2 0.5	0—1.6 0.6
2.2—5.6 3.3	0.4—9.0 3.2	0.2—11.0 3.5	0.2—5.8 3.6	0.2—7.2 4.2	0.2—3.6 2.0
4.2—9.0 6.2	2.6—12.0 7.0	3.5—10.6 6.0	4.4—8.8 7.0	3.4—7.0 5.3	1.0—10.8 4.9
28.2—35.6 32.1	23.8—45.4 34.5	11.0—44.8 33.6	27.6—43.0 32.7	22.0—50.8 31.1	21.0—43.4 32.2
174800—242000 198200	166300—330000 243413	100000—330000 233599	148000—274400 218467	209000—296000 244267	193300—347800 267980
0.3—1.9 1.0	0—1.5 0.5	0.2—1.7 0.5	0.2—1.5 0.6	0.2—1.4 0.7	0.2—1.0 0.4
17—44 29.7	2—17 12	4—65 22.3	3—11 7.7	4—12 8	1—10 5.9

Tabelle 35.

Variationsgrenzen und Mittelwerte der Blutbilder bei der Spät-Nachuntersuchung.

	Bei der Spät-Nachuntersuchung symptomlos 22 Fälle		Bei der Spät-Nachuntersuchung Symptome 8 Fälle		Normalwerte
	Werte vor der Operation	Werte bei der Nachunter- suchung	Werte vor der Operation	Werte bei der Nachunter- suchung	
Hb	63/68—91/98 76/82	62/67—90/97 74/80	65/70—85/92 76/82	64/89—84/91 73/79	75/81—101/109 86/93
Erythroz.	3735—5120 4679	3860—4830 4308	3695—4900 4344	3670—5300 4394	3968—5960 4621
Index	0.68—1.14 0.93	0.83—1.07 0.93	0.78—1.10 0.93	0.81—1.17 0.91	0.84—1.06 0.99
Leukoz.	3850—9600 6575	3800—7800 5492	4900—8600 6645	4750—9100 6319	3800—8900 5975
Neutroph.	44.6—79.6 57.5	39.0—74.5 57.6	48.6—71.0 59.7	54.8—77.8 67.3	47.0—72.0 60.4
Metamyeloz.	0—0.2 0.1	0—0 0	0—0.4 0.1	0—0 0	0—0 0
Stabkernige	0.8—11.2 5.3	0.5—6.8 3.3	2.8—6.2 4.9	3.8—17.2 9.1	1.2—4.6 2.6
Segment- kernige	39.6—65.4 52.2	34.0—74.0 54.3	42.4—64.2 54.7	41.6—71.0 58.2	45.0—68.6 57.8
Basoph.	0—2.0 0.5	0—1.4 0.3	0—1.2 0.4	0—1.8 0.4	0—1.6 0.6
Eosinoph.	0.2—9.0 2.7	0.6—8.2 2.5	0.4—6.2 2.6	0.2—2.4 0.8	0.2—3.6 2.0
Monoz.	2.4—14.2 7.2	1.8—10.6 4.9	3.2—11.2 7.2	2.0—13.0 5.6	1.0—10.8 4.9
Lymphoz.	15.6—46.2 32.1	17.5—47.4 34.5	18.4—39.4 30.2	13.4—36.2 26.0	21.0—43.4 32.2
Thromboz.	148000—380400 227514	134100—330000 203274	155500—265800 219680	166000—244000 207000	193300—347800 267980
Retikuloz.	0.2—1.7 0.6	0.1—1.0 0.3	0.3—1.5 0.8	0.2—0.5 0.3	0.2—1.0 0.4
SR	3—17 13.5	4—13 7.4	6—31 17.3	2—26 13.9	1—10 5.9

trug, in so kurzer Zeit noch nicht ganz normal geworden war. Bei dem Patienten 44 konnte ich keine Ursache zu dem leicht erhöhten Senkungswert feststellen.

Die zweite Gruppe in Tabelle 35 bilden 8 Patienten, bei denen zur Zeit der Nachuntersuchung die früheren Symptome fortbestanden oder die eine zufällige akute Infektion hatten. In den Mittelwerten der Blutbilder dieser Patienten fällt die hohe Zahl der Stabkernigen ins Auge. Ferner lag als auf eine Infektion hinweisendes Zeichen ein etwas übernormaler mittlerer Senkungswert vor, der allerdings niedriger war als vor der Tonsillektomie.

#### d) Besprechung der Ergebnisse.

In den Sternalpunktaten der Tonsillitis chronica-Patienten war die Zellfrequenz, nach den Präparaten zu urteilen, eine ähnliche wie in den Normalfällen. Auch der Erythroblasten-Leukozytenindex stand auf normaler Höhe. Seine Erhöhung kann oft das einzige Zeichen der durch die Infektion verursachten Knochenmarksreaktion sein (KLIMA).

Dagegen zeigten die Punktate qualitative Veränderungen in den gegenseitigen Verhältnissen der Zellen. Sie waren jedoch nicht gross und traten oft nur hervor, wenn man alle Fälle gemeinsam als eine Gruppe betrachtete. Die in den Punktaten angetroffenen Veränderungen waren ähnlicher Art, gleichviel ob die Patienten ausser den durch die chronische Tonsillitis verursachten lokalen Beschwerden noch andere subjektive Symptome oder objektiv feststellbare Krankheiten hatten. Wie früher erwähnt, war auch in der Stärke der Knochenmarks- und Tonsillenveränderungen kein Parallelismus zu entdecken. Mit dem Fehlen eines Parallelismus verhielt es sich also ungefähr wie in den Untersuchungen HOLSTI's über die pathologische Anatomie der Tonsillen bei rheumatischen Infektionen. HOLSTI hat dargelegt, dass auch die Stärke der entzündlichen Prozesse in der Tonsille eine bedeutende Unabhängigkeit von der Intensität der Gelenkaffektionen zeigt. — In 5 Fällen scheint die ausser der Tonsillitis nachgewiesene Zahninfektion keinen Einfluss auf das Mark ausgeübt zu haben.

Vergleicht man die im Zusammenhang mit der akuten und der chronischen Tonsillitis gewonnenen Ergebnisse miteinander, so findet man in ihnen gemeinsame Züge. Erstens zeigte sich im Mark

eine Linksverschiebung der Neutrophilen, die bei der chronischen Tonsillitis nur in bezug auf ihren Grad viel schwächer war als bei der akuten. Zweitens äusserte sich eine Neigung zu Lymphozytose, die am ausgeprägtesten in den Fällen mit Tonsillitis chronica hervortrat. Die Knochenmarksbefunde scheinen demnach die Auffassung zu bestätigen, die heute hinsichtlich der rezidivierenden Tonsillitis herrscht, dass nämlich die Entzündung in den Tonsillen auch während der Intervalle zwischen den akuten Stadien chronisch fort dauert.

Wie bereits erwähnt, habe ich in der früheren Literatur keine Untersuchungen über das Sternalpunktat chronischer Tonsillitis angetroffen. Auch unter den nahe verwandten Krankheitszuständen finden sich nur wenig Vergleichspunkte. FLEISCHACHER und v. LACHNITZ haben das Mark bei 55 Fällen mit Polyarthrits chronica untersucht. Der Zellgehalt der Knochenmarksstrichpräparate war im allgemeinen herabgesetzt. In den gegenseitigen Beziehungen der Leukozyten bestanden keine Abweichungen von der Norm. Die Erythropoese war in keinem einzigen Fall gesteigert. Die Zahl der Plasma- und Retikulumzellen war erhöht. Diese Befunde deuten also teilweise auf eine herabgesetzte Markfunktion hin. In dieser Hinsicht stimmen die Ergebnisse der beiden genannten Forscher einigermaßen mit den meinigen überein, worauf ich später zurückkommen werde. MARKOFF hat die Aufmerksamkeit auf die ausserordentlich empfindliche Reaktion des Knochenmarkes gegenüber jeder Infektion gelenkt. So trat in einem Fall von klinisch symptomlosen tuberkulösem Frühinfiltrat, in dem das Blutbild normal war, im Mark eine Vermehrung der Myelozyten und Stabkernigen sowie ein ungewöhnlich hoher Zellgehalt auf. KLIMA, LABENDZINSKI und ROHR haben bei schweren chronischen Infektionen eine bedeutende Linksverschiebung der Neutrophilen gefunden, wie sie bei meinen eigenen Fällen in leichtem Grade auftrat.

Die Mittelwerte der Blutbilder aus allen meinen Fällen zeigten gewisse Abweichungen von der Norm. Das durchschnittliche Hämoglobinprozent war etwas niedrig. Eine Neigung zu hypochromer Anämie bei Tonsillitis chronica-Patienten haben früher auch ADAMS sowie EDSTRÖM festgestellt.

Die Gesamtzahl der Leukozyten stand auf derselben Höhe wie in den Normalfällen, und die Werte der einzelnen Fälle waren gleich-

mässig auf den normalen Variationsbereich verteilt. Ich konnte also weder eine Neigung zu Leukopenie (DALAND, ADAMS) noch zu Leukozytose (EDSTRÖM) feststellen. Die durchschnittliche Menge der Stabkernigen lag an der oberen Grenze des Normalen, und ausserdem waren bei mehreren Patienten auch Metamyelozyten anzutreffen. Wie aus Tabelle 33 hervorgeht, trat eine Linksverschiebung etwas deutlicher als im Durchschnitt in Fällen auf, in denen sich zu der Tonsillitis eine objektiv feststellbare Begleiterkrankung hinzugesellt hatte. In gleichem Grade wie bei diesen fand sich eine Linksverschiebung auch bei 5 Patienten, die ausser an Tonsillitis an einer Zahninfektion litten, von denen 4 jedoch auch eine objektiv nachgewiesene Sekundärkrankheit hatten. Meine eigenen Beobachtungen bewegen sich in derselben Richtung wie die Feststellungen von GORDING und BJÖRN-HANSEN sowie von SCHWOKOWSKI. Ich konnte bezüglich des Vorkommens der akuten Infektionen keinen Unterschied zwischen den Patienten mit einer Linksverschiebung und den anderen bemerken. Meine Ergebnisse sprechen nicht zugunsten der Ansicht DIAMANT's, dass die Linksverschiebung bei den Tonsillitispatienten ein Zeichen einer zufälligen akuten Infektion wäre und infolgedessen als Kontraindikation der Tonsillektomie dienen könnte. Meines Erachtens stellt die chronische Tonsillenentzündung allein eine hinreichende Ursache für die Entstehung einer solchen Blutreaktion dar. Obwohl wir von einer »Tonsillitis im latenten Stadium« sprechen, wissen wir, dass auch da in den Mandeln ausgedehnte entzündliche Veränderungen bestehen können, die möglicherweise sehr unbedeutende klinische Symptome auslösen, wie auch meine eigenen Fälle zeigen (besonders die Fälle 36, 44, 57 und 65).

In bezug auf die Lymphozyten wiesen meine Ergebnisse nach einer anderen Richtung als die der meisten früheren Forscher. Ihre absoluten und relativen Mittelwerte waren in meinen Fällen dieselben wie bei Normalpersonen. Bei den meisten Untersuchungen, die im Zusammenhang mit einer Fokalinfection ausgeführt wurden, kam man dagegen zu dem Resultat, dass die betreffenden Patienten eine besondere Neigung zu Lymphozytose hatten (Tabelle 36). Nur in wenigen Veröffentlichungen ist angegeben, dass eine solche nicht vorlag (SCHWOKOWSKI, HADEN). Diese Angaben gelten jedoch nur den relativen Lymphozyten-

Tabelle 36.

Die Veränderungen des Blutbildes bei chronischer Tonsillitis.

Autor	Jahr	Zahl der untersuchten Fälle	Veränderungen im Blutbild
DALAND	1921	Nicht ersichtlich	Oft Lymphozytose, zu der sich Leukopenie gesellte. Nach der Tonsillektomie verschwinden die Veränderungen im allgemeinen in 5—8 Wochen.
SCHMIDT	1926	Nicht ersichtlich	Leukozytose zu Zeiten, wenn die Patienten Symptome (Rachenschmerzen, Müdigkeit, Nachtschweiss) haben, obwohl Fieber und Beschleunigung des Pulses fehlen. Die Leukozytose ist ein empfindlicherer Indikator der Infektion als Temperatur und Puls.
ADAMS	1928	Nicht ersichtlich	Neigung zu Anämie, Leuko- und Lymphozytose.
BOLAND	1932	Nicht ersichtlich	In 80 % der Fälle Lymphozytose, die in 4—5 Tagen nach der Tonsillektomie im grössten Teil der Fälle verschwunden ist.
SCHWOKOWSKI	1932	42	In 54.7 % der Fälle Linksverschiebung der Neutrophilen, die einige Monate nach der Tonsillektomie meist verschwunden war. Keine Lymphozytose.
GORDING und BJÖRN-HANSEN	1924—1938	Nicht ersichtlich	Im Blutbild im allgemeinen keine Veränderungen, falls nicht sog. sekundäre Symptome bestehen. Sind sekundäre Symptome vorhanden, dann Linksverschiebung der Neutrophilen und oft Erhöhung der SR. Zwischen Linksverschiebung und SR kein Parallelismus. Bei jungen Leuten, die neben Entzündungsherden rheumatische Symptome haben, im Blutbild Linksverschiebung, aber SR normal. Bei klimakterischen Arthritiden umgekehrt, SR erhöht und weisses Blutbild normal.
DIAMANT	1933	100	1. Bei Tonsillitis im chronischen latenten Stadium, zu der sich sekundäre Symptome gesellt haben, keine Linksverschiebung. 2. Eine Fokalinfection ruft keine Linksverschiebung hervor. 3. Tritt bei chronischer Tonsillitis eine Linksverschiebung auf, so ist sie ein Zeichen einer bestehenden zufälligen akuten Infektion und daher eine Kontraindikation der Tonsillektomie.

Tabelle 36 (Forts.).

Die Veränderungen des Blutbildes bei chronischer Tonsillitis.

Autor	Jahr	Zahl der untersuchten Fälle	Veränderungen im Blutbild
EDSTRÖM	1933	Nicht ersichtlich	Leukozytose, relative Lymphozytose und sekundäre Anämie.
ROSENFELD und LEBEDEFF	1933	100	1. Sowohl bei einfacher chronischer Tonsillitis als in Fällen, zu denen sich sekundäre Krankheiten gesellt haben, ist das Blutbild meist normal. 2. Bei Exazerbation der Tonsillitis Linksverschiebung. 3. Das rote Blutbild weicht nicht von der Norm ab.
THRAHNE	1933	Nicht ersichtlich	Weisses Blutbild im allgemeinen normal. In einem Teil der Fälle nach der Tonsillektomie Lymphozytose, die im Lauf einer Woche nicht verschwunden war.
WESTER- GREN	1933	Nicht ersichtlich	In Fällen von Fokalinfection, in denen die Patienten unbestimmte rheumatische Beschwerden haben, sind das Blutbild und die SR normal oder die Veränderungen sehr unerheblich. Keine Eosinophilie.
KOLBA	1935	100	In 50 % Lymphozytose, in 33 % Neutrophilie, in 8 % normal. In Fällen, in denen Lymphozytose vorlag, waren ausser der Tonsillitis oft sekundäre Krankheiten zu konstatieren.
SCHILLING	1936	Nicht ersichtlich	Das Blutbild zeigt die monozytäre Abwehrphase oder die lymphozytäre Heilphase. Oft Eosinophilie.
VIRKKUNEN	1943	45	Vor der Tonsillektomie: Menge der Erythrozyten normal. Hämoglobinwerte etwas herabgesetzt. Leukozytenzahl normal. Neigung zu Linksverschiebung und zu Monozytose. SR leicht erhöht. Linksverschiebung und Erhöhung der SR am deutlichsten bei Patienten mit Begleitkrankheiten. Nach der Tonsillektomie: 1—2 Tage Zunahme der Leukozytenzahl, der Neutrophilen und der Linksverschiebung und relative Lymphopenie. Während 1—11 Tagen SR gegen vorher erhöht. Spät-Nachuntersuchung: Hämoglobinmenge auf der früheren Höhe. Bei Symptomlosen Werte der Stabkernigen, der Monozyten und der SR normal. Bei Nicht-Symptomlosen die früheren Veränderungen noch vorhanden.



werten, denn in den in Tabelle 36 erwähnten Untersuchungen sind die absoluten Werte nicht berücksichtigt. Früher habe ich darauf hingewiesen, dass meine normalen Lymphozytenwerte verhältnismässig hoch sind. Sie vertreten dieselbe Höhe wie die anderer finnischer Forscher, sind aber etwas höher als die im Schrifttum im allgemeinen als normal betrachteten Werte, wofür allgemein die früher erwähnten Zahlen SCHILLING's gegolten haben. Zieht man dies in Betracht, so dürften zwischen meinen Befunden über die Lymphozyten und den Ergebnissen der früheren Forscher keine bedeutenden Unterschiede bestehen. Als normal sehe ich nämlich Werte an, die die meisten dieser Forscher augenscheinlich als von der Norm abweichend aufgefasst haben.

Als Zeichen eines infektiösen Blutbildes trat in meinen Fällen ferner noch eine Neigung zu Monozytose auf. SCHILLING erwähnt das Vorkommen einer solchen im Zusammenhang mit Fokalinfectionen. Hinsichtlich der Thrombozyten und Retikulozyten war in den Blutbildern nichts Bemerkenswertes zu finden.

Die Senkungsreaktion war bei manchen Patienten erhöht. Das zeigte sich am deutlichsten in Fällen, in denen sich der Tonsillitis Begleitkrankheiten angeschlossen hatten. Leicht erhöhte Senkungswerte wurden aber auch bei mehreren solchen Patienten angetroffen, bei denen ausser der Tonsillitis keine anderen Krankheiten festzustellen waren. Drei von diesen hatten in ihrer Anamnese einige Wochen vorher eine Angina (47, 70 und 74). Es bleiben 6 Patienten übrig (43, 45, 54, 66, 68 und 69), bei denen der erhöhte Wert nur durch die chronische Tonsillitis verursacht sein konnte. Von den letzteren hatten z. B. die Patienten 45 und 68 langandauernde fortgesetzte Schlingbeschwerden, und im Zusammenhang mit der Operation wurden bei ihnen Zeichen einer intensiven Entzündung in den Mandeln gefunden. Derartige Fälle zeigen meiner Meinung nach, dass die chronische Mandelentzündung an sich ohne Begleiterkrankungen eine Erhöhung der Senkungswerte verursachen kann. In dieser Hinsicht stehen meine Ergebnisse im Widerspruch zu den Schlussfolgerungen von LINTZ. Er beobachtete die SR ausser bei chronischer Tonsillitis auch bei anderen fokalen Entzündungsherden. Dabei kam er zu dem Resultat, dass die Herde an sich keine Veränderungen in der Senkungsreaktion bewirken. Wenn man erhöhte Werte antrifft, sind sie seiner Ansicht nach durch eine Begleitkrankheit hervorgerufen. Auch DIAMANT führt die in seinem

Material bei einigen Patienten vorgekommenen erhöhten Werte auf frühere Infektionen in den Anamnesen zurück. EDSTRÖM ebenso wie MUSSARIA sind der Meinung, dass eine erhöhte Senkung zu den Veränderungen gehört, die durch Fokalherde ausgelöst werden können.

Sucht man zu diagnostischen Zwecken im Blutbild und in der Senkungsreaktion nach einer Stütze für einen klinischen Verdacht auf chronische Tonsillitis, so darf man nicht erwarten, grosse Veränderungen zu finden. In dieser Beziehung sind auch geringe Abweichungen vom Normalen zu beachten. In den Blutbildern meiner Fälle lagen meist ein oder mehrere Werte ausserhalb der normalen Variationsgrenze. Völlig normal waren die Befunde nur in 12 Fällen, in 33 dagegen traten in einem oder mehreren Werten unbedeutende Abweichungen auf, die in den verschiedenen Fällen nach verschiedenen Richtungen deuteten, weshalb die Mittelwerte keinen vollen Aufschluss über sie geben. Wie sich aus dem Obigen ergeben hat, gilt dasselbe auch von der Senkungsreaktion. Auch da waren Grenzwerte oder leicht erhöhte Werte gewöhnlich.

Die Tonsillektomie rief in den Sternahpunktaten keine Frühreaktion hervor. Beim Vergleich der Werte der ersten Untersuchung mit den Punktaten aus den ersten Tagen nach der Operation liessen sich in den gegenseitigen Beziehungen der Zellen keine wahrnehmbaren Veränderungen nachweisen. Auf eine mögliche Vermehrung der myeloischen Zellen deutet jedoch die unerhebliche Steigerung des Erythroblasten-Leukozytenindex, die 1—8 Tage nach der Tonsillektomie, aber nicht mehr später auftrat. Der unmittelbare Einfluss der Operation auf das Mark zeigte sich also lange nicht so deutlich wie bei den Granulozytopenie-Anginen, bei denen die grossen Veränderungen in schnellem Tempo verschwanden, und bei der akuten Tonsillitis, bei der die Tonsillektomie eine gelegentliche Verstärkung der von früher bestehenden Linksverschiebung der myeloischen Zellen bewirkte.

In den Blutbildern kam die durch die Operation ausgelöste Reaktion deutlicher als im Marke zum Vorschein, indem sie eine schnell vorübergehende Zunahme der Gesamtmenge der Leuko-

zyten und eine Linksverschiebung der Neutrophilen zeigten. Diese Befunde stimmen mit den früheren Untersuchungen von ANDROLI und CALABRESI überein.

In den Senkungswerten äusserte sich die Einwirkung der Operation ebenso deutlich wie in den Blutbildern. Ein Unterschied bestand nur darin, dass die bei der ersteren festgestellten Veränderungen langsamer als bei der letzteren verschwanden. Meine Ergebnisse bestätigen die Resultate von MORGENROTH, dass der Übergang der SR zur Norm nach der Tonsillektomie lange Zeit in Anspruch nehmen kann.

Bei der Spät-Nachuntersuchung waren bei den nicht symptomlosen Patienten die vor der Tonsillektomie bestehenden Veränderungen sowohl im Marke als im Blutbild und in den Senkungswerten noch vorhanden. Bei den symptomlosen Patienten dagegen waren von den bei der ersten Untersuchung konstatierten hämatologischen Veränderungen manche verschwunden, während einige andere noch festzustellen waren. In den Sternalpunktaten liess sich bei der Nachuntersuchung keine Zunahme der myeloischen Prototypen mehr nachweisen. Der Mittelwert der fertigen Segmentkernigen hatte sich seit der ersten Untersuchung erhöht, obwohl immer noch eine leichte Neutropenie im Vergleich zu den Normalfällen zu erkennen war. In den Blutbildern war die Linksverschiebung verschwunden, und der frühere ziemlich hohe Mittelwert der Monocyten war bei den Spät-Nachuntersuchungen derselbe wie bei den Normalpersonen. Auch der vor der Tonsillektomie leicht erhöhte mittlere Senkungswert war jetzt normal.

Zwischen den zu verschiedenen Zeiten ausgeführten hämatologischen Untersuchungen und dem klinischen Zustand der Patienten herrschte also eine weitgehende Übereinstimmung. Dies zeigt meiner Ansicht nach, dass die Veränderungen in der Myelopoese und der Senkungsreaktion trotz ihres geringen Grades ein Zeichen einer infektiösen Reaktion und nicht von zufälliger Art sind oder durch Ungenauigkeiten der Untersuchungsmethoden bedingt werden. Mit grosser Wahrscheinlichkeit war die festgestellte Reaktion durch die Tonsillitis und in einem Teil der Fälle ausserdem durch die Zahnherde hervorgeru-

f e n. Diese hämatologische Feststellung ist bemerkenswert, da sie zu den objektiv für die Fokalinfectionstheorie sprechenden Untersuchungen gehört, deren es, wie WESTERGREN hervorhebt, im Schrifttum verhältnismässig wenige gibt.

Von Veränderungen, die bei den symptomlosen Patienten nicht nach der Tonsillektomie verschwanden, fand sich im Blutbild eine Neigung zu niedrigen Hämoglobinwerten und zu erhöhten Lymphozytenmengen im Mark. Die durchschnittliche Herabsetzung des Hämoglobinwertes bei den Tonsillitispatienten war nicht gross. Im Verhältniss zu den Normalfällen kam sie jedoch deutlich zum Vorschein. Bei der Spät-Nachuntersuchung ergab sich klinisch keine Ursache, aus der sich der fortgesetzte niedrige Stand der Werte erklärt hätte. Aus diesem Grunde sind sie meines Erachtens als eine Äusserung der andauernd torpiden Funktion der Erythropoese aufzufassen. Hierauf hinweisende Umstände finden sich auch, obwohl undeutlich, in den Sternalpunktaten. Aus der leicht erhöhten oder herabgesetzten Gesamtmenge der Erythroblasten im Differentialbild allein lassen sich keine Schlüsse ziehen, weil auf dasselbe in hohem Grade der Zustand der Myelopoese einwirkt. Bei meinen Patienten wich das Verhältnis der Normo- und Makroblasten etwas vom Normalen ab. Die Menge der Makroblasten war höher als gewöhnlich. Dies ebenso wie die bei allen Untersuchungen aufgetretene etwas geringere Menge der roten Mitosen würde auf eine verlangsamte Erythropoese hindeuten. In den Werten der Retikulozyten sind dagegen ebensowenig im Mark wie im Blut Abweichungen von der Norm festzustellen. Zum Vergleich sei erwähnt, dass KAETHER im Zusammenhang mit der rheumatischen Infektion herabgesetzte Retikulozytenwerte im Mark gefunden hat, und zwar auch dann, wenn die Menge des Hämoglobins und der Erythrozyten normal war.

Die Knochenmarkslymphozytose, die in allen zu verschiedenen Zeiten entnommenen Sternalpunktaten zu konstatieren war, ist früher nicht festgestellt worden. Will man versuchen aufzuklären, worauf diese gesteigerte Menge der Lymphozyten beruht, so kommen zwei Alternativen in Betracht. Die erste Möglichkeit ist, dass die Lymphozyten in dem Punktat aus dem Blut stammen und ihr erhöhter Wert also von der dem Punktat beigemischten Blutmenge herrührt. Die zweite Alternative ist, dass das Mark der Patienten mehr Lymphozyten enthält als das der Gesunden. Ich habe früher

darauf hingewiesen, dass eine Lymphozytose in den Punktatener meiner Patienten die Regel war und nicht nur ganz ausnahmsweise vorkam. Vergleicht man die Mark- und die (absoluten) Blutlymphozytenwerte miteinander, so ist kein Parallelismus festzustellen. Hohe Lymphozytenwerte des Blutes können ebensogut bei hohen als bei niedrigen Werten des Markes auftreten. Die erwähnten Umstände sprechen meiner Ansicht nach dafür, dass die in den Punktatener gefundenen hohen Werte wirklich der Ausdruck einer Lymphozytose des Knochenmarkes sind. Dass die Werte auch nach der Tonsillektomie konstant bleiben, zeigt, dass es sich wahrscheinlich um einen vom Normalen dauernd abweichenden Zustand handelt.

Aus pathologisch-anatomischen Untersuchungen ist schon lange bekannt, dass im Knochenmark ab und zu Lymphknoten auftreten, aus denen die in den Punktatener anzutreffenden Lymphozyten stammen können. ASKANAZY, v. FISCHER, MAYER und FURUTA sowie später WILLIAMS haben sie in 15—60 % der untersuchten Fälle festgestellt. Ob die Lymphknoten zu dem anatomischen Bild des normalen Knochenmarkes gehören oder ob es pathologische Befunde sind, das sind Fragen, die bisher nicht endgültig geklärt sind. Nach den eben genannten Forschern ist ihr Vorkommen als normal, wenn auch nicht als konstant zu betrachten. Das Material von WILLIAMS umfasste u. a. durch Unfall ums Leben gekommene Personen, in deren Anamnese keine entzündlichen Krankheiten aufgetreten waren. Auch im Knochenmark dieser fanden sich Lymphknoten relativ ebenso zahlreich wie in dem anderen Material, woraus er schliesst, dass die Lymphknoten keinen Zusammenhang mit den entzündlichen Krankheiten haben. HELLMAN seinerseits betrachtet sie als metaplastische Veränderungen, die unter dem Einfluss verschiedenartiger toxischer Faktoren entstehen. Zugunsten dieser Auffassung verweist er u. a. auf die Untersuchungen von GLIMSTADT an bakterienfreien Meerschweinchen, deren ganzes lymphatisches System im Vergleich zur Norm ausserordentlich schwach entwickelt war. HALLERMANN hat sie zahlreich bei einem Patienten angetroffen, der lange an einer Polyarthritiser gelitten hatte und dann an einer Granulozytopenie-Angina gestorben war. Da sich die Lymphknoten nicht während der kurzen Krise entwickelt haben konnten, hält er sie für die Folge einer rheumatischen Infektion. Einen ähnlichen Fall hat auch LEUCHTENBERGER veröffentlicht.

ROHR ist auf Grund von gewöhnlichen Strichpräparaten aus Punktkarten und auch von Schnittpräparaten zu dem Ergebnis gelangt, dass Lymphozytenherde im Knochenmark normalerweise nur in Ausnahmefällen vorkommen, viel häufiger aber im Zusammenhang mit bestimmten Krankheiten, von denen vor allem perniziöse Anämie, Granulozytopenie, Panmyelophthie und chronische Infektionen zu nennen sind. Er hat die Auffassung geäußert, die lymphatische Metaplasie entsände ebenso wie die retikuläre oft infolge einer chronischen unspezifischen Knochenmarksentzündung, als deren Ursachen er u. a. chronische Polyarthritis und fokale Entzündungsherde namhaft macht. Die von ihr hervorgerufenen Symptome sind anfangs unbedeutend. Oft erscheinen als ihr einziges Zeichen im Blutbild abnorm niedrige Werte. Die Folge einer solchen Entzündung würde ein sich allmählich entwickelnder Insuffizienzzustand des Markes sein. Wenn das Mark dabei noch von einer zufälligen Noxe, wie einer akuten Infektion, betroffen wird, manifestiert sich die langwierige Markerkrankung in Form abnormer Blutreaktionen, z. B. einer Granulozytopenie.

Die Beobachtungen ROHR's haben mich veranlasst nachzusehen, ob eine erhöhte Lymphozytenmenge in meinen Fällen auf die Funktion des Knochenmarkes einwirkt. Zu diesem Zweck habe ich in Tabelle 37 die Patienten auf Grund der Marklymphozytenwerte in zwei Gruppen eingeteilt. (Bei diesem Vergleich habe ich die Fälle 49, 51, 71 und 72, bei denen sich im Zusammenhang mit der Tonsillektomie Sekundärinfektionen entwickelten, unberücksichtigt gelassen.) Die erste Gruppe bilden 9 Patienten (35, 37, 42, 43, 51, 60, 61, 76 und 77), deren Werte oberhalb der normalen Variationsgrenzen lagen und bei denen die Knochenmarkslymphozytose daher deutlicher hervortrat als bei den anderen Patienten. Klinisch waren bei zwei objektiv eine Nebenkrankheit und bei den anderen subjektive Beschwerden festgestellt worden. In der anderen Gruppe war der Mittelwert der Lymphozyten niedriger, aber doch etwas höher als bei den Gesunden. In den Mittelwerten der Punktkarte und Blutbilder beider Gruppen waren Unterschiede nachzuweisen. Im Knochenmarksbild der ersten Gruppe traten sowohl myeloische als erythroische Zellen spärlicher als in den Normalfällen auf. Unter den Prototypen der Leukozyten war die Verminderung der Metamyelozyten und Myelozyten im Verhältnis am stärksten. Den niedrigen Knochenmarkswerten entsprach im Blutbild eine relativ niedrige

Tabelle 37.

Sternalpunktate und Blutbilder auf Grund der Menge der Knochenmarkslymphozyten in zwei Gruppen geteilt.

		Fälle mit deutlicher Knochenmarkslymphozytose		Andere Patienten	
		Vor der Tonsillektomie 9 Fälle	1—3 T. nach der Tonsillektomie 9 Fälle	Vor der Tonsillektomie 32 Fälle	1—3 T. nach der Tonsillektomie 32 Fälle
Bas. Myel. & Leukoz.	%	0—0.75 0.33	0—0.25 0.1	0—0.75 0.2	0—0.75 0.15
Eos. Myeloz.		0.75—5.0 2.31	0.75—3.0 1.89	0.75—8.50 2.81	0.5—6.75 2.65
Eos. Leukoz.		0.25—4.0 1.36	0—3.0 1.19	0—3.75 0.97	0—3.75 1.17
Myelobl.		0.25—2.75 0.92	0—2.0 1.19	0.5—3.50 1.56	0.25—2.75 1.45
Neutr. Promyeloz.		1.25—9.25 5.03	2.75—11.50 5.86	1.75—14.0 6.54	1.0—14.50 6.60
Neutr. Myeloz.		7.0—17.50 10.41	6.25—19.75 12.44	8.50—23.25 15.55	4.75—27.0 15.72
Neutr. Metamyeloz.		5.0—18.50 11.72	9.50—19.75 14.07	15.50—28.0 19.71	4.75—28.75 17.90
Neutr. stabk. Leukoz.		5.75—11.25 10.14	6.50—13.25 9.67	8.25—21.75 13.95	3.50—17.50 11.74
Neutr. segm. Leukoz.		12.50—42.0 26.47	13.0—44.75 24.92	6.25—37.50 18.05	10.0—45.50 20.70
Lymphoz.		26.75—37.0 30.0	23.0—38.0 26.68	4.0—24.0 18.53	10.0—38.50 20.54
Monozyt.		0.25—4.0 1.67	0.50—3.0 1.67	0—3.0 1.20	0—3.50 1.39
Retikulum- & Plasmaz.	Zahl auf 400 L	2—25 8.67	3—13 8.2	0—19 8.4	1—26 9.09
Megakar.		0—1 0.02	0—0 0	0—2 0.37	0—1 0.13
Normobl.		11—143 60.33	14—105 48.11	34—138 70.1	12—146 61.9
Makrobl.		1—24 7.9	0—16 5.89	1—33 6.9	0—23 6.1
Proerythrobl.		0—12 1.3	0—7 1.1	0—4 0.5	0—3 0.59
Rote Mitosen		0—4 0.89	0—4 1.33	0—4 1.25	0—5 1.16
Weisse Mitosen		0—5 1.1	0—1 0.22	0—4 0.78	0—2 0.5
Retikuloz.		0.4—1.8 0.78	0.8—1.8 1.4	0.5—3.6 1.81	0.6—4.5 1.87

Tabelle 37 (Forts.).

Sternalpunktate und Blutbilder auf Grund der Menge der Knochenmarkslimphozyten in zwei Gruppen geteilt.

	Fälle mit deutlicher Knochenmarkslimphozytose		Andere Patienten	
	Vor der Tonsillektomie 9 Fälle	1—3 T. nach der Tonsillektomie 9 Fälle	Vor der Tonsillektomie 32 Fälle	1—3 T. nach der Tonsillektomie 32 Fälle
Hb	67/72—100/108 79/85	67/72—100/108 79/85	63/68—98/106 76/82	55/59—98/106 75/81
Erythroz.	3695—5600 4576	3920—5850 4621	3930—5120 4536	3070—5160 4443
Index	0.82—1.14 0.95	0.79—1.08 0.93	0.68—1.10 0.91	0.69—1 0.91
Leukoz.	4200—7700 5586	5300—9300 7261	3850—9800 6623	5000—14000 8412
Neutroph.	44.4—68.2 53.7	50.2—78.6 66.7	42.4—79.6 57.8	46.0—88.0 66.4
Metamyeloz.	0—0.2 0.1	0—0.2 0.1	0—0.4 0.1	0—1.6 0.2
Stabkernige	0.6—6.2 2.97	1.0—10.0 4.9	1.0—11.2 5.3	1.0—14.0 5.0
Segmentkernige	41.8—63.0 50.0	44.0—66.0 61.7	36.2—68.4 52.4	36.2—77.6 61.2
Basoph.	0—0.6 0.4	0—1.4 0.4	0—2.0 0.4	0—2.0 0.4
Eosinoph.	0.2—3.8 1.6	0—3.6 1.0	0—9.0 3.0	0—6.8 1.9
Monozyt.	3.0—11.0 8.1	3.0—8.8 5.7	2.4—11.2 6.6	2.0—13.4 6.6
Lymphozyt.	24.6—45.4 36.2	13.6—44.0 26.2	15.6—46.2 32.2	12.8—42.8 24.7
Thromboz.	148000—251200 106433	141100—259600 198671	136500—380400 232053	153500—374300 209067
Retikuloz.	0—1.3 0.5	0.1—0.9 0.4	0—2.6 0.7	0.2—1.9 0.7
SR	2—11 5.7	9—15 11.8	3—47 14.7	3—55 22.6



Leukozytenzahl. Eine Linksverschiebung der Neutrophilen war nicht zu finden. Die Werte des roten Blutbildes waren ziemlich normal und höher als in den Fällen der zweiten Gruppe. Dies kam vor, obwohl im Mark der Patienten der Lymphozytengruppe die Zahl der Retikulozyten und der roten Mitosen niedriger und die der Makroblasten etwas höher als in der zweiten Gruppe war, Umstände, die auf eine herabgesetzte erythropoetische Tätigkeit hinweisen dürften. In den zur zweiten Gruppe gehörenden Punktaten zeigte sich eine Vermehrung der myeloischen Prototypen. Der Mittelwert der Leukozyten war in diesen Fällen um 1000 höher als in der ersten Gruppe. Diese Verschiedenheiten sprechen dafür, dass die leukopoetische Tätigkeit des Markes bei Patienten mit einer beträchtlichen Knochenmarkslymphozytose etwas niedriger wäre als bei den anderen.

Die Tonsillektomie bildete in meinen Fällen eine Art Belastungsprobe, die die Funktionstüchtigkeit des Knochenmarkes weiter beleuchtet. In beiden Gruppen war die postoperative Erhöhung der Leukozytenzahl im Blute gleich gross. Bei der Durchmusterung der Knochenmarkswerte findet man, dass die Tonsillektomie in der ersten Gruppe eine deutliche Linksverschiebung der myeloischen Zellen verursacht hat; in der zweiten Gruppe war keine solche Einwirkung zu bemerken. Die durch den gleichen Reiz ausgelöste, gleich starke periphere Erhöhung der Leukozytenzahl äusserte sich also im Mark der Patienten der Lymphozytosegruppe deutlich in einer stärkeren Reaktion als bei den anderen. Dies zeigt, dass der Gleichgewichtszustand des Knochenmarkes bei den ersteren labiler war als bei den letzteren, obwohl in den Fällen der Lymphozytosegruppe keine deutliche Knochenmarksinsuffizienz festzustellen war.

Im Licht der weiter oben angeführten früheren Untersuchungen ist es wahrscheinlich, dass die Lymphozytose in meinen Fällen der Ausdruck einer lymphatischen Metaplasie des Markes ist. Mit Hilfe von Strichpräparaten aus den Punktaten lässt sich das Vorhandensein einer solchen jedoch nicht beweisen. Nur durch Schnittpräparate könnte ermittelt werden, ob die Zahl der Lymphherde zugenommen hat.

Meine Arbeit klärt nicht die Frage über die Bedeutung der Lymphozytose (oder der möglicherweise hinter ihr liegenden Metaplasie). Nach der Beseitigung des Fokus war die Links-

verschiebung bei der Myelopoese verschwunden, was die klinische Feststellung bestätigt, dass der bei den Patienten herrschende Infektionszustand gleichfalls aufgehört hatte. Nichtsdestoweniger blieb die Lymphozytose während der ganzen Beobachtungszeit unverändert. Wie mir scheint, könnte das ein im Knochenmark auftretendes Zeichen eines allergischen Reaktionszustandes sein. Für die Entstehung eines solchen gibt es mehrere pathogenetische Möglichkeiten. Eine rezidivierende Tonsillitis kann schon an und für sich die Äusserung eines allergischen Zustandes sein. Sie kann aber auch die Ursache zu seiner Entstehung oder Verstärkung darstellen. Ob ein allergischer Zustand im Knochenmark Reaktionen oder Organveränderungen hervorruft, wie er z. B. die rheumatische hyperergische Gelenkentzündung verursacht, wissen wir bisher nicht genau. Die oben angeführten Ergebnisse ROHR's und die Beobachtungen HOLLMANN's über die leukopenische Erschöpfungsphase im Zusammenhang mit bestimmten rheumatischen Infektionen sowie auch meine eigenen Befunde über die Lymphozytose lassen vermuten, dass ein allergischer, hinsichtlich seiner Pathogenese wechselnder Zustand zu Veränderungen des Knochenmarkes führen kann, die instande sind, hemmend auf dessen Funktion einzuwirken. Die Klärung dieser Fragen muss künftigen Forschungen vorbehalten bleiben.

### c) Zusammenfassung.

*Das Sternalpunktat, das Blutbild und die Senkungsreaktion von 45 an chronischer Tonsillitis leidenden Patienten wurde vor der Tonsillektomie, in den ersten Tagen danach und bei 30 Patienten durchschnittlich ein halbes Jahr später untersucht. Es wurden kleine Abweichungen vom Normalen festgestellt, die am deutlichsten in den Mittelwerten hervortraten. Myelopoese: Vor der Tonsillektomie war die Menge der fertigen Neutrophilen im Mark herabgesetzt und waren die Mittelwerte der Prototypen etwas höher als normalerweise. Es zeigte sich zwischen den Patienten kein ausgeprägter Unterschied unabhängig davon, ob sich zu der Tonsillitis Begleiterkrankungen gesellt hatten oder nicht. Im peripheren Blut trat bei normaler Gesamtmenge der Leukozyten eine leichte Neigung zu Linksverschiebung und Monozytose auf. Es kam keine Eosinophilie vor. Die Linksverschiebung war am deutlichsten bei Patienten, die ausser*

der Tonsillitis eine objektiv feststellbare Sekundärerkrankung hatten. In der ersten Zeit nach der Tonsillektomie waren in den Punktionen keine deutlichen Veränderungen im Vergleich mit der ersten Untersuchung zu konstatieren. Im Blut dagegen traten während zweier Tage eine Zunahme der Leukozytenmenge und eine Linksverschiebung der Neutrophilen auf. Bei der Spät-Nachuntersuchung waren bei den symptomlosen Patienten (22 Fälle) im Mark und Blut die Linksverschiebung verschwunden und die Monozytenwerte des Blutes auf die Norm herabgegangen. Die Patienten, bei denen die Infektionssymptome fortbestanden (8 Fälle), wiesen im Mark und Blut ähnliche Veränderungen auf wie bei der ersten Untersuchung. Erythropoese: In den zu verschiedenen Zeiten entnommenen Sternalpunktionen zeigte sich eine mässige Neigung zu erhöhten Makroblastenwerten und einer herabgesetzten Zahl der Mitosen. Im peripheren Blut war die Menge der Erythrozyten bei allen Untersuchungen normal, aber die Mittelwerte des Hämoglobins waren etwas erniedrigt. Retikuloendothel: Die Menge der Retikulum- und Plasmazellen in den Punktionen wich nicht von der normalen ab. Thrombopoese: Die Zahl der Megakaryozyten im Mark und die Menge der Thrombozyten im Blut waren dieselben wie in den Normalfällen. Lymphopoese: Die Mittelwerte der Lymphozyten im Mark waren bei allen Untersuchungen erhöht und blieben fortlaufend konstant. Die Lymphozytenwerte des Blutes wichen bei den verschiedenen Untersuchungen nicht von der Norm ab. Bei Patienten, bei denen die Knochenmarkslymphozytose am stärksten war, war die Menge der myeloischen ebenso wie die der erythroischen Zellen im Mark niedriger als normalerweise und die Leukozytenzahl des Blutes durchschnittlich um 1000 niedriger als bei den anderen Patienten. Die Tonsillektomie löste bei diesen im Mark eine myeloische Reaktion aus, die bei den anderen nicht zum Vorschein kam, während die Leukozytosereaktion des peripheren Blutes in beiden Gruppen die gleiche war. Senkungsreaktion: Der mittlere Senkungswert war vor der Tonsillektomie leicht erhöht, am deutlichsten bei den Patienten mit Begleiterkrankungen. Die Tonsillektomie bewirkte einen Anstieg der Senkungswerte, der während der ersten 11 Tage, aber nicht mehr später zu beobachten war. Zur Zeit der Spät-Nachuntersuchung war die SR bei den symptomlosen Patienten normal, bei denen mit Symptomen leicht erhöht.

#### IV. ZUSAMMENFASSUNG.

Es wurden das Sternalpunktat und das Blutbild vor der Tonsillektomie und danach bei Fällen mit Angina granulocytopenica, Tonsillitis acuta und Tonsillitis chronica untersucht. Als Kontrollmaterial dienten 20 subjektiv und objektiv gesunde Personen.

1. Mit Hilfe von 6 Fällen sollte die Frage weiter beleuchtet werden, auf welche Weise die während des akuten Stadiums ausgeführte Tonsillektomie auf den Verlauf der Granulozytopenie-Angina einwirkt. Einer der Patienten (25), dessen Sternalpunktat und Blutbild zeigten, dass eine weit entwickelte myeloische Aplasie vorlag, starb, während der frühere septische Zustand unverändert fortdauerte, 24 Stunden nach der 8 Tage nach dem Ausbruch der Krankheit ausgeführten Tonsillektomie. Bei der Obduktion wurde eine Pneumonie festgestellt. In einem anderen Fall (26) verschwanden die schweren hämatologischen Veränderungen zuerst nach der 6 Tage nach dem Ausbruch der Krankheit erfolgten Operation, rezidierten aber bald, wobei 3 Tage nach der Tonsillektomie bei der Patientin eine Pneumonie konstatiert wurde und sich bei ihr ausserdem eine zu einer Ösophagusfistel führende Mediastinitis entwickelte. Die Patientin starb 11 Tage nach der Tonsillektomie. 4 andere Patienten, von denen zwei (21 und 24) nach Ausweis der hämatologischen Veränderungen und des klinischen Krankheitsbildes eine schwere und die beiden anderen (22 und 23) eine leichte Krise hatten, genasen schnell nach der 3—12 Tage nach dem Ausbruch der Krankheit ausgeführten Operation. Abgesehen von der Tonsillektomie wurden 3 Patienten mit einer geringen Anzahl von Leberinjektionen und 4 mit Sulfanilamidpräparaten behandelt. Die Remission des Blutes erfolgte im Lauf von 2—5 Tagen und nach der Auffassung, die der Verf. aus dem Schrifttum erhalten hat, schneller, als es in den konservativ behandelten Fällen zu geschehen pflegt. Die Heilung der Operationswunden war, abgesehen von dem zuerst erwähnten zum Exitus gegangenen Fall, normal. Nach dem plötzlichen Verschwinden des Fiebers

und der hämatologischen Veränderungen zu urteilen, wurde die Remission durch die Tonsillektomie gefördert. Von der Art ihrer Einwirkung auf die Aufhebung des Krisenzustands des Blutes kann man sich eine Vorstellung bilden, wenn man den Einfluss der Tonsillektomie auf das Mark und das Blut bei der gewöhnlichen akuten Angina vergleicht. Im Lichte der letzterwähnten Fälle kann man schliessen, dass die Operation bei den Granulozytopenie-Anginen auf den hämatologischen Zustand teilweise wie ein unspezifischer Reiz, hauptsächlich aber wie eine spezifische Massnahme einwirkt, die durch die Beseitigung des Infektionsherdes eine Aufhebung des Krisenzustands zur Folge hat. Auf Grund der Fälle betrachtet der Verf. die Tonsillektomie bei den Granulozytopenie-Anginen in solchen Fällen als möglich und indiziert, in denen ausser der Tonsillitis keine anderen entzündlichen Herde bestehen und das Sternalpunktfat zeigt, dass eine Regeneration des Blutes möglich ist. Als ätiologische Ursachen der Krise kamen in den geheilten Fällen ausser den infektiösoxischen Momenten konstitutionelle Faktoren und Medikamente in Betracht. Im Hinblick darauf hält es der Verf. für wahrscheinlich, dass die Tonsillektomie ausser in Fällen, in denen die Tonsillitis primär ist, auch in Fällen von Nutzen sein kann, in denen sie sekundär ist. Bei der Behandlung der Granulozytopenie-Angina ist die Tonsillektomie nicht immer allein eine hinreichende therapeutische Massnahme, sondern da ist es angebracht, ihren Effekt durch eine die Hämatopoese stimulierende Behandlung und möglicherweise durch Sulfanilamidpräparate zu unterstützen. Die bisherige Erfahrung lehrt, dass die Tonsillektomie in der Therapie der Granulozytopenie-Angina von grosser Bedeutung ist, was mehr Beachtung verdient, als es bisher im Schrifttum der Fall gewesen ist.

2. Der Einfluss der akuten Tonsillitis und der während derselben ausgeführten Tonsillektomie auf die Hämatopoese wurde in 6 Fällen untersucht. Vor der Operation zeigte das Knochenmarksbild eine myeloische Hyperplasie, eine Linksverschiebung der myeloischen Zellen und eine Vermehrung der Retikulumzellen, also Veränderungen, die man im allgemeinen bei akuten Infektionen antrifft. Dagegen war die Menge der Lymphozyten im Mark grösser, als es bei akuten Infektionen gewöhnlich ist. Wie der Verf. annimmt, beruht dies darauf, dass es sich in den Fällen von Tonsillitis acuta nicht um eine einfache akute Infektion, sondern um das akute Stadium einer chronischen Tonsillenerkrankung handelte. In den Blutbildern trat eine

neutrophile Leukozytose auf, an die sich eine leichte Linksverschiebung anschloss. In den ersten Tagen nach der Tonsillektomie war die infektiöse Reaktion des Knochenmarkes noch stärker als vorher und die Linksverschiebung im peripheren Blut gesteigert. Die Veränderungen des Blutbildes verschwanden nach der Tonsillektomie schnell, obwohl nicht so schnell wie das Fieber. Aus dem Sternalpunktat verschwanden die Veränderungen langsamer, und sie waren teilweise noch 10 Tage nach der Tonsillektomie vorhanden.

3. Bei 45 Patienten mit chronischer Tonsillitis wurden das Sternalpunktat und das Blutbild vor der Tonsillektomie, während der ersten Tage danach und in 30 Fällen überdies durchschnittlich ein halbes Jahr später untersucht. Klinisch bildete das Material eine fortlaufende Serie von Patienten (6 Fälle), die ausser der Tonsillitis keine andere Krankheiten oder Beschwerden hatten, sowie ferner von Fällen (25 Fälle) mit verschiedenartigen subjektiven Nebensymptomen (Müdigkeit, unbestimmten »rheumatischen« Schmerzen) und drittens Patienten (14 Fälle), bei denen auch objektiv eine Begleiterkrankung (Polyarthrit, Nephritis usw.) festzustellen war.

Die Zellfrequenz der Sternalpunktate wich bei den Tonsillitis chronica-Patienten ebensowenig von der Norm ab wie der Erythroblasten-Leukozytenindex. Vor der Tonsillektomie zeigten die Mittelwerte der Punktate im Vergleich zu den Normalwerten eine Verminderung der fertigen Neutrophilen, eine leichte Zunahme der myeloischen Prototypen und eine Erhöhung der Lymphozytenzahl. Bei den erythroischen Zellen trat eine leichte Neigung zu erhöhten Makroblastenwerten und einer herabgesetzten Zahl der Mitosen auf. Zwischen den Patienten bestand kein deutlicher Unterschied mit Rücksicht darauf, ob sich der Tonsillitis die obenerwähnten Nebensymptome oder -krankheiten angeschlossen hatten oder nicht. Die Mittelwerte der Blutbilder aus allen Fällen zeigten eine etwas herabgesetzte Hämoglobinnmenge sowie bei normaler Leukozytenzahl eine leichte Linksverschiebung und eine Neigung zu Monozytose. Die Lymphozyten waren nicht vermehrt. Bei den Normalpersonen war der relative Mittelwert der Lymphozyten 32.2 % und der absolute 1924. Die Senkungsreaktion war durchschnittlich etwas höher als normalerweise. Die Linksverschiebung und die Erhöhung der SR waren am ausgeprägtesten bei den Patienten mit Begleitkrankheiten.

Die Tonsillektomie verursachte in den folgenden Tagen nach der Operation keine deutliche Reaktion im Mark. Doch weist die während der

ersten Tage auftretende leichte Erhöhung des Erythroblasten-Leukozytenindex auf eine myeloische Hyperplasie hin. In den Blutbildern zeigte sich an 2 Tagen nach der Tonsillektomie eine Zunahme der Leukozytenzahl, der Neutrophilen und der Linksverschiebung sowie eine relative, aber nicht absolute Lymphopenie. In den Senkungswerten rief die Operation eine etwa 10 Tage andauernde Erhöhung hervor.

Bei der Spät-Nachuntersuchung, durchschnittlich ein halbes Jahr nach der Tonsillektomie, waren bei den Patienten, die immer noch Infektionssymptome hatten (8 Fälle), die früheren Veränderungen sowohl im Mark als im Blut und in den Senkungswerten vorhanden. Bei den symptomlosen Patienten (22 Fälle) waren die Veränderungen zum Teil verschwunden und zum Teil konstant geblieben. Im Mark fand sich ebensowenig wie im Blut eine Linksverschiebung; auch der frühere ziemlich hohe Monozytenwert im Blut war bei der Spät-Nachuntersuchung auf dasselbe Niveau wie in den Normalfällen zurückgegangen. Der Mittelwert der Senkungsreaktion war normal. Die vollständige Übereinstimmung zwischen den klinischen und hämatologischen Befunden beweist nach der Ansicht des Verf., dass die Veränderungen in der Leukopoese und den Senkungswerten der Ausdruck einer von der chronischen Tonsillitis ausgelösten Reaktion sind. Sie geben der Fokalinfectionstheorie eine objektive Stütze.

Die Neigung zu niedrigen Hämoglobinwerten des Blutes und zu einer erhöhten Lymphozytenmenge des Markes waren bei den symptomlosen Patienten Veränderungen, die bei der Nachuntersuchung ähnlich wie bei der ersten Untersuchung hervortraten. Die konstant niedrigen Hämoglobinwerte betrachtet der Verf. mit Rücksicht auf die oben beschriebenen leichten Veränderungen in den Werten der erythroischen Zellen des Markes als Äusserung einer andauernd torpiden erythropoetischen Funktion.

Die Erhöhung des Mittelwertes der Lymphozyten im Mark war bei den zu verschiedenen Zeiten ausgeführten Untersuchungen gleich gross. Der Verf. sieht in ihr eine Äusserung einer wirklichen Knochenmarkslymphozytose und führt sie am ehesten auf eine metaplastische Markveränderung zurück. Er spricht die Vermutung aus, dass ihr ein im allergischen Sinn veränderter Reaktionszustand der Patienten zugrunde liegen könnte. An der Pathogenese derselben kann u. a. eine rezidivierende Tonsillitis beteiligt sein. Es wurde versucht, festzustellen, ob die erhöhte Marklymphozytenmenge auf die Funktion des Knochenmarkes einwirkt. Dabei erwies es sich, dass bei

*der Gruppe von Patienten, bei denen die Marklymphozytenwerte deutlich oberhalb der normalen Variationsgrenze lagen (9 Fälle), sowohl die Menge der myeloischen als der erythroischen Zellen im Mark herabgesetzt und die Leukozytenzahl im peripheren Blut um 1000 kleiner war als bei den anderen Patienten. Die »Belastungsprobe«, die die Tonsillektomie darstellte, verursachte bei der ersteren Gruppe im Blut eine gleich grosse Erhöhung der Leukozytenzahl wie bei den letzteren. Im Mark dagegen trat in den Fällen der Lymphozytosegruppe eine deutliche Linksverschiebung der myeloischen Zellen auf, die bei den anderen Patienten nicht festzustellen war. Der Gleichgewichtszustand des Knochenmarkes war also bei den Patienten der Lymphozytosegruppe labiler als in der anderen Gruppe.*



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# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CXLVII

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DAS  
DIABETESPROBLEM  
DIE RHYTHMISCHEN  
STOFFWECHSELVORGÄNGE

VON  
JAKOB MÖLLERSTRÖM

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STOCKHOLM 1943



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# DAS DIABETESPROBLEM



DAS  
DIABETESPROBLEM

DIE RHYTHMISCHEN  
STOFFWECHSELVORGÄNGE

VON

JAKOB MÖLLERSTRÖM

VORSTAND DER B. A. HJORTHSCHEEN ABTEILUNG FÜR STOFFWECHSELFORSCHUNG  
AM WENNER-GREN-INSTITUT DER UNIVERSITÄT STOCKHOLM

*Herausgegeben mit Unterstützung  
des Längmanschen Kulturfonds*

---

STOCKHOLM 1943

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STOCKHOLM 1943  
SVENSKA TRYCKERIAKTIEBOLAGET

*Berndt August Hjorth*

*in memoriam*



## V O R W O R T

Als ich mich vor vier Jahren dazu entschloss, die Erfahrungen meines damals 10jährigen Diabetesstudiums in einer orientierenden Einführung in das Diabetesproblem niederzulegen, war ich von dem lebhaften Gefühl geleitet, dass die periodischen Stoffwechselprozesse, wie wir ihnen im Leberhythmus und dem endogenen neurohormonalen Wechselspiel begegnen, auf fundamentale Vorgänge des biologischen Geschehens im Organismus zurückzuführen sein müssen. Nur mit Ausgangspunkt hiervon ist es möglich, sich Einblicke in das Diabetesproblem und die Pathophysiologie der Stoffwechselstörungen zu verschaffen, welche dem Arzt Wege zu einer rationelleren Therapie öffnen, als klinische Untersuchungsmethoden, die vielfach auf periphere Erscheinungen abzielen, den inneren Ursachenzusammenhang aber oft unberücksichtigt lassen.

Es war keine leichte Aufgabe, in gedrängter Form das Wesentliche aus einer fast unüberschbaren Fülle von Erfahrungstatsachen herauszuschälen, wie sie das Schrifttum auf den verschiedensten Gebieten enthält. Noch schwieriger war es, dieselben mit eigenen Beobachtungen unter Berücksichtigung des endogenen Rhythmus zu einem Ganzen zu verschmelzen, um dadurch ein erschöpfendes Bild der Diabeteslehre mit ihren Problemen zu erhalten. Der Unvollständigkeit der vorliegenden Arbeit bin ich mir wohl bewusst — es ist ja nicht möglich, einer orientierenden Schrift den Umfang eines Handbuchs zu geben —, hoffe aber gleichzeitig, dass dieselbe doch in gewissem Masse dazu beitragen möge, den Gesichtskreis des praktisch tätigen Kliniklers im Sinne eines tieferen Verständnisses für die Stoffwechselkrankheiten und die äusserst komplizierten Verhältnisse zu erweitern, welchen man beim Studium von Störungen im chemischen Ablauf der Lebensprozesse gegenübersteht. Jede schablonenmässige Diabetesbehandlung ist da ausgeschlossen. Kann die Arbeit überdies Impulse zu weiterer systematischer Forschung geben, welche vielleicht



in Zukunft die grossen Lücken unserer Kenntnisse über Pathogenese und rationelle Therapie der Stoffwechselkrankheiten ausfüllen wird, dann ist das Ziel derselben erreicht.

Die Vollendung der vorliegenden Arbeit wäre mir unmöglich gewesen, wenn ich nicht dank glücklicher Umstände im Laufe der Jahre den unschätzbaren Vorteil gehabt hätte, mit einer Reihe von Spezialforschern auf dem Gebiete der physiologischen Chemie und der biologischen Stoffwechselforschung in enger Verbindung zu stehen. Gross ist meine Dankesschuld gegenüber Herrn Prosektor *Hjalmar Holmgren* und Herrn Professor *Ragnar Nilsson* für vieljährige gemeinsame Arbeit und anregenden Gedankenaustausch, welche im Laufe der vergangenen Jahre sich als für das klinische Diabetesstudium in hohem Masse förderlich erwiesen haben. Das gleiche gilt für die Herren Professoren *Karl Myrbäck*, *Hugo Theorell* und *Henrik Lundegårdh* sowie Dozent *Sven Forssman*, jeder auf seinem Spezialgebiet. Ein besonderer Dank gebührt Herrn Professor *Hugo Theorell*, der mir bei der Ausarbeitung des fünften Abschnitts wertvolle Hilfe geleistet hat, sowie Herrn Professor *Ragnar Nilsson*, welcher den vierten Abschnitt der Arbeit durchsah. In Dankbarkeit wende ich mich auch an meine Kollegen im Wenner-Gren-Institut für experimentelle Biologie der Universität Stockholm, die Herren Professor *John Runnström*, Dozenten *Åke Öhrström*, *Lennerstrand*, *Gunnar Ågren* und *Ehrensward*. Namentlich will ich noch an dieser Stelle meinen nächsten Mithelfern bei der täglichen Arbeit, den Herren Dr. *Olov Lindberg* und *Thor Lövgren*, meinen aufrichtigen Dank darbringen. Alle haben mit ihren Erfahrungen auf verschiedenen Wissenschaftsgebieten dazu beigetragen, der gemeinsamen Arbeit im Forschungsinstitut die Atmosphäre der Vielseitigkeit zu verleihen, welche notwendig ist, damit die Grenzgebiete einander begegnen und die klinische Erfahrung durch Berührung mit den schwer zugänglichen Grundlagen der physiologischen Chemie und Zellphysiologie vertieft werde.

Bei einem eingehenderen Studium der Augenveränderungen in dem klinischen Diabetesmaterial ist mir der Ophthalmologe Dr. *Yngvar Lindblad* behilflich gewesen, bei der Beurteilung nervöser Symptome die Neurologen Dr. *Torsten Frey* und Dr.

*Gunnar Ahlberg* sowie in bezug auf den kindlichen Diabetes der Pädiater Dozent *J. Henning Magnusson*.

Schliesslich gebührt Herrn Chefarzt Dozent *Erik Forsgren* die Ehre, den 24-Stundenrhythmus zuerst bei der Glykogenspeicherung beobachtet und damit dem Studium der endogenen Rhythmik des biologischen Kohlehydratumsatzes eine feste Unterlage gegeben zu haben.

Stockholm, im Dezember 1942.

*Jakob Möllerström.*



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## I. ABSCHNITT

## Einleitende Bemerkungen zur allgemeinen Pathogenese des Diabetes.

Nachdem *Thomas Willis* 1674 bei gewissen Fällen von Polyurie einen süssen Geschmack des Harns festgestellt und *Dobson* und *Cowley* durch Eindickung des Urins Zucker erhalten hatten, welcher, wie später von *Höme* gezeigt wurde, sich als von Hefe vergärbar erwies, begann sich ein Krankheitsbegriff mehr und mehr herauszukristallisieren, welcher von *Rollo* 1797 als »Diabetes mellitus«, Zuckerharnruhr, bezeichnet wurde. Man verstand unter ihm eine chronisch verlaufende Krankheit, deren vornehmstes Merkmal die Zuckerausscheidung durch den Harn war. Daneben konnten eine Reihe klinische Symptome auftreten, Polyurie, Heiss hunger, Trockenheit der Haut, Magenbeschwerden, Augenveränderungen, Affektionen des Herzens, der Nerven und des Gefässsystems usw., sämtlich mit einer langsam fortschreitenden Kachexie Hand in Hand gehend.

*Rollo* äusserte 1798 die Ansicht, der Diabetes mellitus beruhe auf einer Magenerkrankung. Diese werde dadurch verursacht, dass Vegetabilien mehr als es normalerweise der Fall ist in Zucker umgewandelt und im Harn ausgeschieden werden. Einen Beleg für diese Anschauung fand *Rollo* in seiner diätetischen Therapie, bei der eine strenge Fleischkost sowie Opiate zur Dämpfung der Intensität der Verdauungstätigkeit die Zuckerdiurese herabsetzten. *Nicolas* und *Gueudeville* bezeichneten 1803 die Zuckerkrankheit als »Phthisurie sucrée«. Sie hielten den Zustand für auf einer Darmerkrankung mit abnormer Bildung eines zuckerhaltigen Darminhalts beruhend, wodurch ein zuckerreicher und stickstoffarmer Chylus entstehe, der den Organismus nicht hinreichend zu ernähren vermöchte und dadurch zur Abmagerung führe. Eine reichliche

animalische Diät wurde als notwendig erachtet, um dem Stickstoffmangel zu begegnen.

*Chevreuil* (1815) wies nach, dass der Diabetikerurin Traubenzucker oder Glykose enthält. Diese ist ein normales Produkt beim Abbau der Stärke im Laufe der Verdauung. *Gmelin* und *Tiedemann* fanden auch 1826, dass das Blut normalerweise Traubenzucker enthält. Beim Diabetes stellte *Ambrosiani* 1835 einen erhöhten Traubenzuckergehalt des Blutes fest.

Die Lehre von der abnormen Zuckerbildung im Darmkanal konnte daher nicht richtig sein, und die Theorie über eine enterogene Ursache des Diabetes musste fallengelassen werden.

Der erhöhte Zuckergehalt des Diabetikerblutes weckte schon früh Aufmerksamkeit. *Mialhe* stellte 1839 die Theorie auf, dass eine herabgesetzte Alkaleszenz des Diabetikerblutes eine auf verminderter Glykolyse beruhende Anhäufung von Zucker im Blute verursache. Er ging von der Beobachtung aus, dass Traubenzucker bei Gegenwart von Alkali leichter oxydiert wird und verschwindet. Die geringere Alkaleszenz war nach *Mialhe* auf eine Exkretionsstörung mit Ansammlung von sauren Produkten im Blut zurückzuführen. Der sekundär angehäuften Zucker im Blut werde dann durch die Nieren ausgeschieden. Die Behandlung sei nach *Mialhe* in erster Linie so einzurichten, dass der Alkalimangel kompensiert und dadurch die Glykolyse gesteigert werde. *Mialhe* führte als erster Alkali in die Diabetestherapie ein.

### Die Entdeckung des Glykogens

Eine neue Epoche der Diabetesforschung wurde durch eine Reihe von Entdeckungen *Claude Bernards* eingeleitet. Dieser hatte 1848 gemeinsam mit *Barreswil* nachgewiesen, dass die Leber, unabhängig von der Art der Nahrung, mehr Zucker enthält als irgendein anderes Organ. Nur bei lange anhaltender Nahrungskarenz wird die Leber nahezu zuckerfrei. *Baumert* und *Frerichs* konnten diese Entdeckung bestätigen. Einige Jahre später zeigte *Claude Bernard*, dass der höchste Zuckergehalt im Blute aus der Vena hepatica vorlag, auch wenn im Portablut nur geringe Zuckermengen zu finden waren.

Eine neue Entdeckung machte *Claude Bernard* 1855. Bei Versuchen an Tieren mit Durchtrennung des Rückenmarks fand er bei der Obduktion fast zuckerfreie Lebern. Wurden diese zuckerarmen Lebern einige Stunden unberührt liegen gelassen, so war bisweilen ein stetiges Steigen des Zuckergehalts derselben zu konstatieren.

*Claude Bernard* setzte das Verschwinden des Leberzuckers in Beziehung zum Sinken der Körpertemperatur nach der Rückenmarksdurchtrennung. Er wies nach, dass die postmortale Zuckerbildung in der Leber durch Abkühlung gehemmt wurde, bei Erwärmung aber wiedereintrat.

Aus seinen Beobachtungen zog *Claude Bernard* den Schluss, dass sich in der Leber eine Vorstufe des Traubenzuckers befinden müsse. Nach einer Reihe systematischer Untersuchungen konnte *Claude Bernard* im März des Jahres 1857 die Entdeckung des Leberglykogens oder der tierischen Stärke veröffentlichen und die Methode zur Reindarstellung desselben angeben.

Bald nach der *Claude Bernardschen* Entdeckung des Leberglykogens gelang es *Sanson*, Glykogen in der Muskelsubstanz nachzuweisen. Später ist Glykogen in den verschiedensten Geweben und Organen gefunden worden.

Die grosse Verbreitung des Glykogens im Tierreich ist ein Hinweis darauf, dass dasselbe eine für den Stoffwechsel des lebenden Organismus ausserordentlich wichtige Substanz darstellt. Die hier eingefügte Zusammenstellung nach *Schöndorff* macht die Verteilung des Glykogens im Organismus sowie die Schwankungen desselben beim Hund ersichtlich.

Organ	Glykogen in Prozent						
	Tier 1	Tier 2	Tier 3	Tier 4	Tier 5	Tier 6	Tier 7
Blut . . . . .	0,0046	0,0015	—	—	0,0061	—	—
Leber. . . . .	4,354	7,6021	18,69	17,096	16,375	9,89	7,297
Muskel . . . .	0,7195	0,8778	2,5406	3,233	3,7217	2,526	0,7599
Knochen . . .	0,183	0,3925	0,9963	1,3129	1,7635	0,9729	0,2736
Därme . . . .	0,0254	0,0753	1,4674	1,5144	1,7162	1,01	0,2024
Netz . . . . .	0,3755	0,1999	0,726	0,839	1,5974	0,9151	0,0859
Herz . . . . .	0,118	0,0995	0,5791	0,7153	1,2073	0,4939	0,231
Gehirn . . . .	0,0435	0,2283	0,266	0,227	0,1964	0,254	0,1984

Die grossen Schwankungen im Glykogenvorrat des Organismus beruhen zum Teil auf dem Ernährungszustand des letzteren, lassen sich aber nicht durch wechselnde Nahrungszufuhr allein erklären. *Forsgren* hat im Jahre 1927 nachgewiesen, dass der Glykogengehalt der Leber grossen Veränderungen unterworfen ist, abhängig von einem 24stundenperiodischen Wechsel der Leberfunktion und teilweise ohne Beziehung zur Nahrungszufuhr. Diese rhythmische Lebertätigkeit mit abwechselnd Bildung von Gallenbestandteilen und Speicherung von Glykogen ist ein für die Menge des Leberglykogens mitbestimmender Faktor.

### Die Ketonkörperbildung beim Diabetes

Bei gewissen Fällen von Zuckerkrankheit nimmt die Ausatemungsluft einen eigentümlichen, fruchtartigen Geruch an. Dieser Geruch beruht, wie *Petters* 1857 fand, auf dem Vorkommen von Azeton in der ausgeatmeten Luft. Gleichzeitig kommt Azeton im Blut und Urin vor. Die Bildung des Azetons führte *Petters* auf eine abnorme Zuckergärung im Magen-Darmkanal zurück. *Cantani* konnte indessen nachweisen, dass der Magendarminhalt bei Azetonurie fast frei von Azeton war. Das Azeton konnte daher nicht im Darmkanal gebildet werden. Dagegen zeigte *Cantani* 1877, dass Blut aus der V. hepatica reicher an Azeton war als solches aus der V. portae. Es war infolgedessen wahrscheinlich, dass das Azeton in der Leber entsteht.

Im Jahre 1865 stellte *Gerhardt* fest, dass azetonhaltiger Harn mit Eisenchlorid eine Farbreaktion gibt. *Ebstein* und *Tollens* (1881) konstatierten, dass die bei der *Gerhardtschen* Reaktion wirksame Substanz nur in saurer Lösung extrahierbar ist und mithin eine Säure sein muss. *V. Jaksch* (1881) und *Deichmüller* (1882) konnten die gesuchte Säure in Form ihres Kupfersalzes als Diazetsäure identifizieren. Diese zerfällt leicht in Azeton und Kohlendioxyd.

Die Farbreaktion mit Eisenchlorid wird von der Enolform der Diazetsäure verursacht.

*Hallervordens* fand 1880, dass der Urin bei vielen Fällen von Zuckerharnruhr mit positiver Eisenchloridreaktion Ammoniak in beträchtlich gesteigerter Menge enthielt, mehr als den damals bekannten organischen Säuren im Urin entsprach. Es musste daher eine bisher unbekannte Säure in entsprechender Menge vorhanden sein, welche das Ammoniak neutralisiert. Beim Suchen nach dieser Säure entdeckte *Stadelmann* 1883 die Krotonsäure. Diese war jedoch nicht die gesuchte Säure, sondern ein Umwandlungsprodukt der  $\beta$ -Oxybuttersäure, welche *Minkowski* und — unabhängig von diesem — *Külz* 1884 in Urin mit hohem Ammoniakwert nachweisen konnten. Ausser bei gewissen Diabetesformen tritt  $\beta$ -Oxybuttersäure noch bei Fieber und zahlreichen Infektionskrankheiten (*Külz* 1884), bei Darmstörungen mit heftigem Erbrechen und Inanition sowie bei Skorbut (*Minkowski* 1885) und anderen krankhaften Zuständen auf.

Azeton, Diazet- oder Azetessigsäure und  $\beta$ -Oxybuttersäure stehen in enger Beziehung zueinander. Sie können ineinander umgewandelt werden, und man fasst sie daher nach *Geelmuyden* als Azeton- oder Ketonkörper zusammen.

Normalerweise kommen nur Spuren von Ketonkörpern im Blute vor, Azeton-Diazetsäure bis 0,15 mg%,  $\beta$ -Oxybuttersäure bis 1,5 mg% (nach *Engfeldt*). Beim Diabetes kann der  $\beta$ -Oxybuttersäuregehalt des Blutes auf mehrere hundert mg% steigen. Die normale Ausscheidung von Ketonkörpern entspricht 10—20 mg im Urin und 20—89 mg in der Ausatemungsluft, als Azeton pro 24 Stunden berechnet.

Ketonkörper treten daher auch beim normalen Stoffwechsel in geringen Mengen auf. *Minkowski* (1893) hielt sie für eine Zwischenstufe bei der Kohlehydratbildung aus Fett. Beim Phloridzindiabetes fand er nämlich im Tierversuch eine geringfügige Steigerung der Zuckerausscheidung, wenn Azetonkörper, Buttersäure oder Kapronsäure subkutan injiziert wurden.

Die Bedeutung der Fette für die Ketonkörperbildung geht aus Arbeiten von *Geelmuyden*, *Magnus-Levy*, *Landergren*, *Knoop*, *Embsen* u. a. hervor. Bei Kohlehydratmangel in der Nahrung verbrennt der Organismus sein eigenes Reservefett.

Im Hungerzustand nimmt deshalb die Ketonkörperbildung zu. Dies macht sich besonders bei gewissen Diabetesformen mit herabgesetzter Glykogenbildungsfähigkeit der Leber bemerkbar.

Die Frage der Ketonkörperbildung wird im II., IV. und VI. Abschnitt unter verschiedenen Gesichtspunkten erörtert werden.

### Das Eingreifen des Nervensystems in den Zuckerstoffwechsel

Mit der Entdeckung des Leberglykogens begann eine neue Ära der Diabetesforschung. Im Jahre 1857 machte *Claude Bernard* noch eine für die Diabeteslehre grundlegende Entdeckung: er stellte den Einfluss des Nervensystems auf den Kohlehydratstoffwechsel fest.

Bei Versuchen an Kaninchen fand *Claude Bernard*, dass ein Stich in den Boden des vierten Ventrikels zwischen Acusticus- und Vagus Kern eine starke Glykosurie und Polyurie zur Folge haben konnte.

Der Lokalisation des »Zuckerstichs« entspricht ein von *Owsjannikow* entdecktes vasomotorisches Zentrum. Von diesem Zentrum in der Medulla oblongata aus verlaufen vasomotorische Bahnen teils im N. vagus und teils als absteigende Fasern im Rückenmark. Die vasomotorischen Nerven der Leber finden wir einerseits im Vagus, andererseits verlassen diese das Rückenmark in der Lumbalregion und ziehen durch die Nn. hepatici weiter. Vasomotorische Nervenfasern gehen auch vom oberen Thorakalmark aus, passieren den sympathischen Grenzstrang und erscheinen dann in den Nn. splanchnici. Sie sammeln sich zum Plexus coeliacus und begleiten die Art. hepatica zur Leber. *Ein Reizzustand in diesem vasomotorischen System kann einen Diabetes auslösen.*

Das Auftreten von Glykosurie beim Zuckerstich hängt nach *Cyon* und *Aladov* mit einer Gefässerweiterung und veränderter Durchblutung der Leber zusammen. Man kann sich daher vorstellen, dass eine Glykosurie auch bei einer veränderten Durchblutung anderen Ursprungs zustande kommt, z. B. beim Versagen der Herzkraft.

Je mehr Glykogen die Leber enthält, desto stärker wird die durch den Zuckerstich verursachte Glykosurie. Bei hungernden Tieren mit wenig Leberglykogen hat der Zuckerstich nur eine geringe Wirkung.

Schon *Claude Bernard* hatte demonstriert, dass der Effekt des Zuckerstichs ausbleibt, wenn der N. splanchnicus major auf beiden Seiten durchtrennt worden war. *Cavazzani* konnte ferner zeigen, dass eine Reizung von sowohl Nn. splanchnici als auch Plexus coeliacus einen erhöhten Zuckergehalt in der V. hepatica bewirkt. *Eckhard* fand, dass mechanische Reizung des ersten Thorakalganglions eine Glykosurie verursacht, welche verschwindet, wenn das Ganglion entfernt wird.

Die Wirkung des Zuckerstichs besteht jedoch auch dann weiter, wenn die Nn. vagi durchtrennt werden.

Bereits *Claude Bernard* konstatierte eine gesteigerte Zuckerdiurese bei Reizung des zentralen Teils eines durchtrennten Vagus.

Auch Reizung anderer zentripetal leitender Nerven, z. B. des N. depressor, N. ischiadicus u. a., kann eine vermehrte Zuckerbildung zur Folge haben. Dies muss auf dem Wege über zentrale Reflexbahnen stattfinden, wobei zur Leber und anderen Organen ziehende sekretorische und vasomotorische Nervenbahnen den Impuls vermitteln.

*Eckhard* bestätigte 1869, dass Vagusreizung eine Glykosurie hervorruft. Nach Durchschneidung der Nn. vagi und Reizung des peripheren Vagusabschnitts stellten *Leven* und *Butte* eine Zunahme des Blutzuckers in der V. hepatica fest. Dies liess sich auch an hungernden Tieren mit glykogenarmer Leber nachweisen. *Die Vagusreizung scheint daher unabhängig vom Glykogengehalt der Leber eine vermehrte Zuckerbildung in derselben auslösen zu können.* *Leven* und *Butte* nahmen an, dass in der Nähe des Vaguszentrums ein spezielles Zuckerzentrum liegt, dessen Reizung eine von vasomotorischen Veränderungen in der Leber unbeeinflussbare Zuckerausscheidung bewirkt. Sie führten diese Zuckerausscheidung auf eine gesteigerte Zuckerbildung aus Eiweiss zurück, da die Stickstoffausscheidung gleichlaufend mit der Zuckerausscheidung bei Vagusreizung zunahm.



*Frédérique* und *Nuel* beobachteten von Änderungen der Zirkulationsverhältnisse in der Leber unabhängige Schwankungen des Glykogengehalts derselben. Sie nahmen daher eine direkte Beteiligung des Nervensystems bei der Glykogenbildung an. *Gürber* wies an Kaninchen nach, dass die Leber während des Winters eine Glykogenmenge enthielt, die bis dreimal so gross war wie im Sommer, und zwar trotz einheitlicher Fütterung; er hielt dies für den Ausdruck eines nervös gesteuerten Anpassungsmechanismus.

Gewisse Diabetesformen fasste *Paschutin* als das Resultat einer diabetischen, von trophischen nervösen Störungen verursachten Gewebs- und Organdegeneration auf. Einer gesteigerten Ausscheidung von sowohl Harnstoff wie Zucker kann ein vermehrter Eiweisszerfall zugrunde liegen. *Paschutin* macht auf die grundsätzliche Übereinstimmung dieser Diabetesform mit der Fettsucht aufmerksam. Im ersten Falle entsteht bei dem abnormen Eiweissabbau Glykose als stickstofffreies Produkt, im letzteren Neutralfett, welches infolge seiner Unlöslichkeit in Zellen und Geweben abgelagert wird.

### Kohlehydratstoffwechsel und innere Sekretion

Der Einfluss des Nervensystems auf den Zuckerstoffwechsel steht in enger Beziehung zur Funktion der Einsonderungsdrüsen.

Wenn die Nn. splanchnici gereizt werden entsteht eine Hyperglykämie. *Christie*, *Macleod* und *Pearce* (1911) haben gefunden, dass diese Hyperglykämie nach Exstirpation der Nebennieren ausbleibt.

*Blum* hatte schon 1901 festgestellt, dass eine Einspritzung von Nebennierenextrakt eine starke Blutzuckersteigerung und Glykosurie hervorrief. Die dabei wirksame Substanz, das *Adrenalin*, ist das zuerst rein dargestellte und seiner Konstitution nach bestimmte Hormon. Das Adrenalin mobilisiert hauptsächlich das Muskelglykogen und bewirkt dadurch eine hochgradige Blutzuckerzunahme (vgl. S. 43).

*Stewart* und *Rogoff* zeigten, dass der Zuckerstich beim Ka-

ninchen auch nach Entfernung jeglichen Nebennierengewebes eine Hyperglykämie verursacht. *Zuckerstich und Splanchnicusreizung sind infolgedessen im Hinblick auf ihre hyperglykämisierende Wirkung nicht gleichwertig.* Der Zuckerstich löst im Gegensatz zur Splanchnicusreizung auch nach Nebennierenexstirpation eine Hyperglykämie aus.

*Macleod und Pearce* haben beobachtet, dass, wenn die zur Leber führenden Nervenbahnen unterbrochen werden, die Nebennieren und ihre Nervenverbindungen aber unversehrt sind, auch die hyperglykämisierende Wirkung der Splanchnicusreizung ausbleibt. Es ist daher nicht die vermehrte Adrenalinbildung als solche, welche bei der Splanchnicusreizung die Hyperglykämie verursacht, sondern der Effekt wird von Nervenbahnen der Leber vermittelt. *Macleod und Pearce* nehmen an, dass der Glykogenabbau unter nervöser Kontrolle steht und von der Nebennierenfunktion und Adrenalinbildung abhängig ist. *Nach Macleod steigert das Adrenalin den Tonus des sympathischen Nervensystems, und es kommt zu einer Wechselwirkung zwischen dem Nervensystem und der endokrinen Funktion der Nebennieren bei der Regelung des Zuckerstoffwechsels.* Diese Frage wird im VI. Abschnitt weiterhin behandelt werden.

Es war bereits *Bouchardat* (1851) aufgefallen, dass bei Diabetes oft Veränderungen im Pankreas vorhanden waren. Dies wurde von vielen Autoren bestätigt.

*Klebs* versuchte 1864, im Tierversuch durch Pankreasexstirpation Diabetes hervorzurufen, erhielt aber keine sicheren Resultate. *Mering und Minkowski* konnten dagegen 1889 bei Versuchen an Hunden nachweisen, dass nach Entfernung der Bauchspeicheldrüse ein schwerer Diabetes mit Glykosurie, Polyurie, rascher Abmagerung und Tod im Koma auftrat.

*Hédon und Minkowski* (1892) zeigten, dass sich der Diabetes verhindern liess, wenn ein unterbundenes Pankreas bei dem operierten Hund subkutan implantiert wurde. Die Genese dieses Diabetes konnte daher keinen Zusammenhang mit der äusseren Sekretion der Bauchspeicheldrüse haben. Da ferner die subkutan implantierte Drüse völlig ohne Nervenverbindungen war, konnten auch keinerlei Nervenimpulse von die-

ser Drüse aus vermittelt werden. Die einzige Möglichkeit war die, dass das Pankreas eine chemisch wirkende Substanz enthält, welche das Zustandekommen des Diabetes verhindert. *Laguesse* (1893) nahm an, dass diese wirksame Substanz in den von *Langerhans* 1869 entdeckten Zellinseln gebildet wird. Diese waren bei Diabetes oft atrophisch und in geringerer Anzahl vorhanden.

*Schulze* (1900) und *Ssobolew* fanden, dass das sekreterzeugende Pankreasgewebe nach Unterbindung des Ductus pancreaticus atrophisch wurde, dass aber die Langerhansschen Inseln hierbei unverändert blieben. Es entstand da auch kein Diabetes, woraus sich ergab, dass die wirksame Substanz sich in diesen Zellinseln befinden muss.

Bereits 1908 war der schwedische Forscher *Paul Sjöquist* auf den Gedanken gekommen, aus der Bauchspeicheldrüse die den Diabetes verhindernde Substanz zu isolieren. In demselben Jahre gelang es *Zuelzer*, aus dem Pankreas ein Krämpfe auslösendes Präparat mit sonst unbekannten Wirkungen darzustellen. Zu dieser Zeit gab es keine brauchbaren Methoden für die Beobachtung der Schwankungen des Blutzuckerspiegels. Pankreaspräparate mit blutzuckersenkender Wirkung haben später *Scott* (1912), *Lesser* (1914) und *Paulesco* (1921) erhalten. Der letztere schlug den Namen *Pankrein* für das wirksame Hormon vor. Gleichzeitig und unabhängig hiervon hatten *Banting* und *Best* (1921) aus unterbundenen Bauchspeicheldrüsen ein bei Diabetes wirksames Hormon dargestellt, welches sie nach *Schafer* (1916) als *Insulin* bezeichneten.

Spritzt man bei Pankreasdiabetes Insulin subkutan ein, so beginnt der Blutzuckergehalt zu sinken, die Polyurie und Ketonurie sowie sonstige Diabetessymptome verschwinden. Das Insulin greift mithin stark in die pankreasdiabetische Stoffwechselstörung ein. Die Blutzuckersenkung erfolgt auch unter normalen Verhältnissen, bei Abwesenheit pankreasdiabetischer Erscheinungen. Hierdurch ist die Möglichkeit gegeben, Insulinpräparate zu standardisieren.

Nicht nur die Bauchspeicheldrüse, sondern auch der Zwölffingerdarm ist von Bedeutung für den Kohlehydratstoffwechsel. *De Renzi* und *Reale* hatten bereits 1890 bei Versuchen an

Hunden nachgewiesen, dass eine Glykosurie entstehen konnte, wenn das Duodenum entfernt, das Pankreas aber zurückgelassen wurde. Auch *Pflüger* (1908) beobachtete Glykosurie nach Exstirpation des Duodenum und fand, dass diese Glykosurie oft einen periodischen Verlauf mit zuckerfreien Tagen aufwies.

Der duodeno-pankreatische Einfluss auf den Zuckerstoffwechsel wird im VI. Abschnitt des näheren besprochen werden.

Neben der Bauchspeicheldrüse sind noch andere innersekretorische Organe von Bedeutung für den Kohlehydratstoffwechsel. Ein Organ, welches hierbei eine zentrale Rolle spielt, ist die Hypophyse.

Nachdem *Pierre Marie* (1886) bei Akromegalie regelmässig eine Hyperplasie oder Adenombildung im vorderen Hypophysenlappen beobachtet hatte, machte *Borchard* (1908) darauf aufmerksam, dass die Akromegalie oft von einem Diabetes begleitet wird. Er versuchte auch, doch ohne Erfolg, Diabetes durch Injektion von hauptsächlich aus dem Hinterlappen hergestelltem Hypophysenextrakt hervorzurufen. Ähnliche Versuche mit negativem Ergebnis wurden 1911 von *Cushing*, *Goetsch* und *Jacobsohn* angestellt. Es gelang dagegen *John*, *O'Mulveny*, *Potts* und *Laughton* (1927), nach Injektion eines Extrakts aus gefrorenen Hypophysenvorderlappen bei Hunden einen transitorischen Diabetes nachzuweisen.

Weitere Untersuchungen haben ersichtlich gemacht, dass die Hypophyse auf mannigfache Art in den Intermediärstoffwechsel eingreift. *Houssay* (1929) beobachtete, dass ein nach Pankreasexstirpation entstandener Diabetes verschwand, wenn die Hypophyse entfernt wurde. Wurde ein Hypophysenvorderlappen bei dem Versuchstier implantiert, oder wurde ein Extrakt aus Hypophysenvorderlappen eingespritzt, so trat von neuem ein Diabetes auf, evtl. in schwererer Form, mit Glykosurie und Ketonkörperausscheidung. Auch die Insulinempfindlichkeit war da gesteigert. Der Vorderlappen der Hypophyse muss daher in den Kohlehydratstoffwechsel und in die Blutzuckerregulation eingreifen. Die Wirkung desselben ist indessen keine einfache, sondern es handelt sich um eine komplexe Reaktion mit Beteiligung mehrerer Hypophysenhormone. Ein

Teil dieser Hormone greift direkt in den Intermediärstoffwechsel ein und regelt u. a. die Zuckerbildung aus Eiweiss und Fett, andere beeinflussen die Glykogenbildung und den Zerfall des Glykogens. Neben diesen Stoffwechselregulatoren gibt es noch eine andere Gruppe von Hypophysenhormonen, die *adenotropen*, welche sich nicht direkt am Stoffwechsel beteiligen, dagegen aber die Hormonerzeugung anderer innersekretorischer Drüsen stimulieren oder hemmen und dadurch den Intermediärstoffwechsel beeinflussen.

Young (1937) hat gezeigt, dass Injektionen von Vorderlappenextrakt bei Hunden einen *Dauerdiabetes* hervorrufen können (Abb. 1). Richardson und Young (1938) fanden, dass die Zellinseln im Pankreas bei diesen diabetischen Hunden verändert wurden, die Zellen derselben entarteten, und die Inseln wiesen nach einer gewissen Zeit eine hyaline Umwandlung auf.

Das diabetesauslösende Hypophysenhormon, welches energisch in den Kohlehydratstoffwechsel eingreift, ist nach Bom-

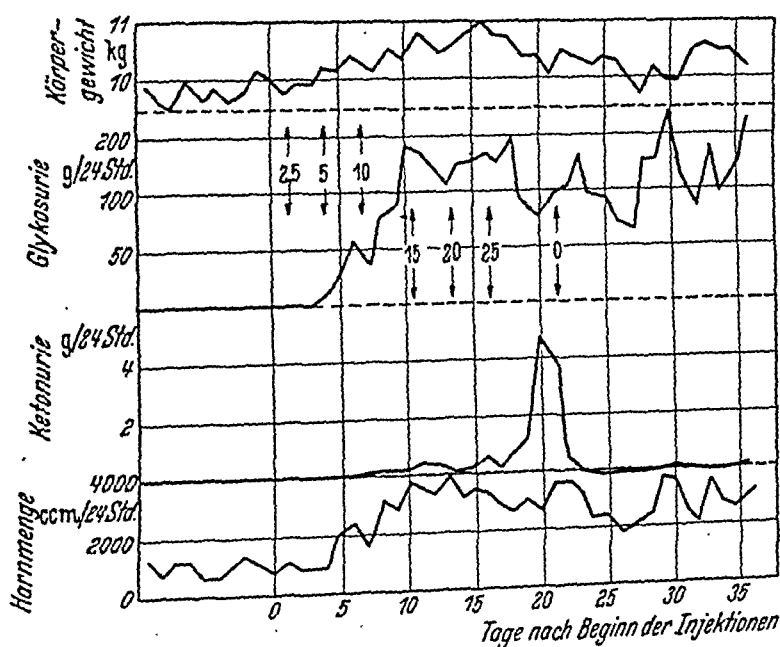


Abb. 1. Erzeugung eines Dauerdiabetes durch Injektion von Hypophysenvorderlappenextrakt. Die Zahlen an den Pfeilen geben die Menge von frischem Vorderlappen in Gramm an, die zur Extraktbereitung benutzt wurde. Tägliche Injektionen bis zum 21. Tag (nach Young).



## II. ABSCHNITT

# Glykogenbildung und Fettstoffwechsel.

Das Glykogen spielt im tierischen und menschlichen Organismus beim Kohlehydrathaushalt der lebenden Zellen eine ausschlaggebende Rolle. Es sind daher schon früh zahlreiche Untersuchungen über das Vorkommen des Glykogens angestellt worden.

*Claude Bernard* hielt die Leber für ein Depotorgan, in welchem Nahrung, die der Körper nicht unmittelbar auszunützen gezwungen war, in Form von Glykogen gespeichert wird. In den ersten Jahrzehnten nach der Entdeckung des Glykogens war deshalb die Aufmerksamkeit in hohem Masse auf die Bedeutung der Nahrung für die Glykogenbildung gerichtet. Viele Untersuchungen wurden vorgenommen, um zur Klarheit über diese Frage zu gelangen.

## Glykogenbildung aus Nahrungsstoffen

### Glykogen und Kohlehydratnahrung

*Pavy* fütterte Hunde teils mit eiweissreicher, animalischer und teils mit kohlehydratreicher, vegetabilischer Kost. Bei 11 Tieren mit Eiweissfutter schwankte das Verhältnis Lebergewicht : Körpergewicht zwischen 1 : 33 und 1 : 30. Die Lebern enthielten bei diesen Tieren im Mittel 7,19 % Glykogen. Wurden die Tiere dagegen mit kohlehydratreicher Kost gefüttert, so betrug die entsprechende Verhältniszahl 1 : 15 bis 1 : 10, und die Lebern enthielten im Mittel 17,23 % Glykogen.

Diese Untersuchungen zeigen eindeutig, dass *kohlehydratreiche Nahrung die intensivste Glykogenbildung bewirkt.*

Das Material zur Bildung des Leberglykogens wird dem Organismus direkt oder indirekt mit der Nahrung zugeführt.

*Polimanti* hat an Hunden nachgewiesen, dass nach Fütterung mit kohlehydratreicher Kost das Blut der *V. portae* reicher an Glykogen ist als das der *V. cava*. *Polimanti* fand

in der <i>V. portae</i>	96,75 mg Glykogen,	
» » <i>V. carotis</i>	58,21 »	»
» » <i>V. cava abdominal.</i>	34,25 »	» in 100 cem Blut.

Dies deutet darauf hin, dass die Glykogenbildung schon in der Darmwandung erfolgen kann, dass aber die Speicherung in der Leber stattfindet. *Arnold, Lang* u. a. haben auch gezeigt, dass das Epithel der Darmschleimhaut Glykogen in geringer Menge enthält. Bei der Kohlehydratesorption wird aus der Darmschleimhaut ein zucker- und glykogenreiches Blut abtransportiert, und die wesentliche Glykogenspeicherung geht dann in der Leber während der assimilatorischen Tätigkeitsphase derselben vor sich.

*Claude Bernard* hatte bereits beobachtet, dass intravenös eingespritzter Rohrzucker restlos im Urin ausgeschieden wurde. Saccharose kann mithin nicht direkt im Organismus umgesetzt oder als Glykogen gespeichert werden. Ebenso verhalten sich viele andere Zuckerarten: Galaktose, Laktose, Maltose, Pentosen usw. Nur Glykose und Laevulose lassen sich ohne weiteres zur Glykogenbildung des Körpers verwerten. Diese Zuckerarten werden daher unter normalen Verhältnissen bei parenteraler Zufuhr in mässiger Menge nicht ausgeschieden.

Polysaccharide können nur insofern, als sie durch die Verdauungstätigkeit in Glykose oder Laevulose übergeführt werden, direkt an der Glykogenbildung teilnehmen.

Der Glykogengehalt der Leber schwankt auch bei reichlicher Kohlehydratzufuhr innerhalb weiter Grenzen.

*Külz* fütterte Tauben 6 Tage lang mit reichlichen Mengen Weizen und Brot. Die Tiere wurden getötet, während die Verdauung im Gange war, und die Lebern unverzüglich auf ihren Glykogengehalt untersucht. Trotz des einheitlichen Tiermaterials und identischer Fütterungsbedingungen fand *Külz* 1891, dass das Leberglykogen der Tiere zwischen 0,91 und 8,89 %



wechselte. Zahlreiche Autoren haben ähnliche Beobachtungen über den schwankenden Glykogengehalt der Leber gemacht. Man muss daher die grösste Vorsicht walten lassen, wenn es sich darum handelt, aus Fütterungsversuchen Schlüsse zu ziehen. Bevor man überhaupt irgend etwas schliessen kann, muss man wissen, innerhalb welcher Grenzen der Glykogengehalt der Leber normalerweise variiert, und welchen Gesetzen diese Variationen folgen.

## Glykogen und Eiweissnahrung

Die Frage, ob aus Eiweiss Glykogen entstehen kann, hat den Gegenstand vieler Untersuchungen gebildet. Eine grosse Fehlerquelle bei allen Fütterungsversuchen mit tierischem Eiweiss ist das Vorkommen von Kohlehydraten in Muskulatur und Geweben, entweder in Form von Glykogen, oder als Glykoproteide.

V. Mering nahm Fütterungsversuche an Hunden vor, welche er 21 Tage hungern liess, worauf sie 4 Tage lang reichlich gut ausgewaschenes Ochsenfibrin zu fressen bekamen. Als dann die Leberglykogenmenge nach Tötung der Tiere bestimmt wurde, betrug diese bei dem mit Eiweiss gefütterten Tier 16,3 g und 0,48 g bei dem hungernden Kontrolltier. Dieser Versuch beweist jedoch nichts über Leberglykogenbildung aus zugeführtem Eiweiss. *Pflüger* fand nämlich bei ähnlichen Hungerversuchen an Hunden noch nach 28tägiger Nahrungsentziehung erhebliche Variationen im Glykogengehalt der Leber und in einem Fall sogar 22,5 g Glykogen, ohne dass irgendwelche Nahrung zugeführt worden war! Das Gewicht des Versuchstiers war dabei von 44 auf 33 kg gesunken. Eine kritische Überprüfung vorangehender Versuche und eigene Arbeiten führten *Pflüger* zu der Ansicht, die Fütterung mit Eiweiss bewirke bei Abwesenheit von Kohlehydraten keine sichere Bildung von Leberglykogen bei normalen Tieren. Bei Kohlehydratmangel muss daher nach *Pflüger* der Glykogenvorrat des Tierorganismus aus Fett ergänzt werden, entweder aus Fett in der Nahrung, oder aus Fettdepots.

Auch beim schwersten Hunger können die Fettdepots nicht

völlig geleert werden. *Sandmeyer* und auch *Pflüger* haben nachgewiesen, dass die Muskulatur bei pankreaslosen Hunden bis 2,6 % Fett enthalten kann, wenn auch die Tiere bis fast zu Gerippen abgemagert waren. Bei normalen Tieren in hochgradigem Inanitionszustand kann die Muskulatur erhebliche, zwischen 0,8 und 9,4 % schwankende Fettmengen enthalten.

Im Gegensatz zur mangelnden Glykogenspeicherung der Normaltiere bei Eiweisszufuhr steht die vermehrte Zuckerausscheidung, wenn bei schwerem Diabetes kohlehydratfreie Eiweissnahrung zugeführt wird.

*Lüthje* beobachtete einen pankreasdiabetischen Hund, welcher 3 Wochen lang mit kohlehydratfreiem Eiweiss gefüttert wurde. Während dieser Zeit wurden 1176 g Zucker im Urin ausgeschieden. Der Gesamtvorrat an Glykogen bei diesem Hund wurde beim Beginn des Versuchs auf 232 g, 257 g Zucker entsprechend, berechnet. Einen Beleg für die Annahme, dass Zucker aus Eiweiss gebildet wurde, fand *Lüthje* in einem konstanten Verhältnis zwischen den ausgeschiedenen Mengen Glykose und Stickstoff.

*Tschannen* (1914) hat gezeigt, dass die Leber bei Ratten, welche mit Pepton gefüttert worden waren, praktisch glykogenfrei ist. Die Glykogenbildung bei gleichzeitiger Pepton- und Kohlehydratzufuhr ist da ebenfalls beträchtlich herabgesetzt, was auf eine Störung in der Tätigkeit der Leberzellen hindeutet.

Es ist also schwer, sich an Hand von Fütterungsversuchen ein klares Bild von der Bedeutung des Eiweisses als Glykogenquelle zu machen.

*Emden* und seine Mitarbeiter haben durch Leberperfusionsversuche nachgewiesen, dass gewisse Aminosäuren, namentlich Alanin, Asparagin, Zystin und Glykokoll, in Glykose umgewandelt werden können. Fütterung mit diesen Aminosäuren hat auch bei pankreasdiabetischen Hunden eine gesteigerte Zuckerausscheidung zur Folge. Die folgende Tabelle S. 18 nach *Emden* und *Salomon* in verkürzter Form macht das Resultat eines derartigen Fütterungsversuchs ersichtlich.

Diese Untersuchungen machen wahrscheinlich, dass das Eiweiss nach Abbau desselben teilweise direkt in Kohlehydrate

Verfütterte Substanz	Zuckerausscheidung		
	am Tage vor dem Fütterungsversuch	am Versuchstage	am Tage nach d. Fütterungsvers.
Alanin.....	16,00 g	29,3 g	19,3 g
» .....	6,2 »	19,5 »	3,6 »
» .....	2,50 »	18,9 »	6,2 »
Asparagin .....	1,7 »	8,48 »	2,45 »
Glykokoll .....	1,79 »	10,05 »	2,45 »
» .....	2,45 »	5,26 »	7,0 »
» .....	2,45 »	7,9 »	3,0 »

(nach Embden und Salomon)

umgewandelt werden kann. Gewisse Aminosäuren sind zuckerbildend, andere nicht. Zu den *glykoplastischen Aminosäuren* rechnet man: Glykokoll, Alanin, Aminobuttersäure, Arginin, Ornithin, Zystin, Asparaginsäure, Glutaminsäure und Histidin. Sind diese Aminosäuren im Eiweiss enthalten, so muss dieses in entsprechendem Grade Glykogen bilden können.

*Richardson* hat durch Perfusionsversuche mit zuckerhaltiger Ringerscher Lösung die Wirkung von Eiweiss und Abbauprodukten desselben in bezug auf die Glykogenbildungsfähigkeit der Schildkrötenleber untersucht. Er fand, dass ein Zusatz von Kasein die Glykogenbildung nicht beeinflusste, welche dagegen von hydrolysiertem Kasein und Pepton gehemmt wurde. Gewisse Aminosäuren, z. B. Glykokoll und Alanin, üben bei gleichzeitigem Vorhandensein von Kohlehydraten keinen Einfluss auf die Glykogenbildung aus, andere wiederum, beispielsweise die Glutaminsäure, wirken nach *Richardson* stark hemmend.

Es ist bemerkenswert, dass eiweissreiche Kost bei Fütterungsversuchen an Normaltieren nicht in entsprechendem Grade zu einer Zunahme des Leberglykogens führt, obgleich Eiweiss bei Diabetestieren eine gesteigerte Zuckerdiurese bewirkt. Dieser Sachverhalt deutet darauf hin, dass die Zuckerbildung im Organismus nicht notwendigerweise mit einer Glykogenspeicherung in der Leber Hand in Hand geht. Zuckerbildung und Glykogensynthese sind, wenigstens unter gewissen Umständen, zwei voneinander getrennte Prozesse.

## Glykogen und Fettnahrung

Von den beiden Komponenten des Neutralfetts ist das Glycerin zweifellos eine Glykogenquelle: Weiss hatte schon 1873 nachgewiesen, dass die Leber bei Hühnern, welche mit Glycerin gefüttert worden waren, bis 36mal so viel Glykogen enthalten konnte wie die Lebern der Kontrolltiere. Luchsinger fand dasselbe Verhältnis bei Kaninchen. In dem diabetischen Organismus geht das Glycerin in Glykose über, was zuerst von Cremer 1902 beim Phloridzindiabetes und von Luthje 1904 beim Diabetes pankreasloser Hunde demonstriert worden war. Da die Glycerinkomponente ungefähr  $\frac{1}{10}$  des Neutralfettmoleküls ausmacht, kann dieser Teil des Fetts ohne weiteres in Glykose umgewandelt werden.

Die Fettsäuren können nicht in entsprechendem Grade direkt in Zucker verwandelt werden und Glykogen bilden. Die Zufuhr von Fettsäuren bewirkt daher beim Diabetes keine sichere Zunahme der Zuckerausscheidung.

Indirekt können dagegen unter normalen Verhältnissen Fettsäuren in Glykogen übergehen, und diese Umwandlung findet möglicherweise auf dem Wege über eine intermediäre Ketonkörperbildung statt. Da indessen über Glykogenbildung aus Fettsäuren nichts Näheres bekannt ist, wird der Fettsäureumsatz im Zusammenhang mit der Ketonkörperbildung (S. 36) behandelt werden.

Die Zuckerbildung aus Fett hängt wahrscheinlich mit dem Lipoidstoffwechsel zusammen (vgl. S. 31).

## Die rhythmische Funktion der Leber

Die bei Fütterungsversuchen beobachteten Schwankungen im Glykogengehalt der Leber lassen sich nicht durch Variationen der Nahrungszufuhr oder äusserer Umstände erklären. Sie waren daher lange ein Rätsel. Erst im Jahre 1927 bewies Erik Forsgren, dass diese Schwankungen des Glykogengehalts der Leber auf einer 24stundenperiodisch wechselnden Lebertätigkeit beruhen, welche teilweise von der zugeführten Nahrung unabhängig ist.

Zum Studium der Gallenkapillaren hatte *Forsgren* 1919 eine histologisch spezifische Gallenfärbungsmethode ausgearbeitet. Diese gründet sich darauf, dass gewisse Gallenbestandteile mit Bariumchlorid unlösliche Verbindungen bilden. Die ausgefällte Galle kann dann nach *Mallory* gefärbt werden, wodurch die Gallenkapillaren sich bis in ihre feinsten Verzweigungen verfolgen lassen. *Forsgren* fand an in dieser Weise behandelten Präparaten, dass die Leber, wenn sie reichlich Glykogen enthielt, arm an Gallenbestandteilen war und vice versa (Abb. 5, S. 25). Hier liegen offenbar zwei alternierende Prozesse vor, Glykogenspeicherung und Gallenbildung, welchen zwei getrennte Phasen der Tätigkeit des Leberparenchyms entsprechen: die assimilatorische und die dissimilatorische. *Forsgren* fand zwei Glykogenmaxima, um 2 Uhr bzw. um 16 Uhr. Zwischen diesen lag je ein Minimum, um 10 Uhr und um 18 Uhr. Die früher bei Fütterungsversuchen konstatierten Schwankungen des Glykogengehalts erhalten durch diesen funktionellen Leberrhythmus ihre natürliche Erklärung.

Die Gallenbildung erfolgt abwechselnd mit der Glykogenspeicherung. Hierdurch bekommt auch die Gallensekretion einen 24stundenperiodischen Verlauf, der teilweise von der Nahrungsaufnahme unabhängig ist.

Die periodische Gallenbildung war bereits 1889 von *Copemann* und *Winston* nachgewiesen worden, deren Befunde u. a. von *Dastre* 1890 und *Pfaff* und *Balch* 1897 bestätigt wurden.

In Abb. 2 wird eine Gallensekretionskurve nach *Pfaff* und *Balch* wiedergegeben.

*Josephson* und *Larsson* (1934) bestimmten die Gallensäuren-

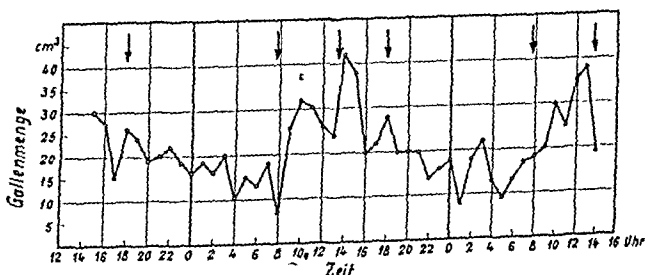


Abb. 2. Entleerung von Galle aus einer dichten Gallenfistel bei einer operierten 38jährigen Frau. Die Pfeile geben die Mahlzeiten an (nach *Pfaff* und *Balch*).

menge bei einem Patienten mit Gallenfistel und fanden, dass die Gallensäuren im grossen ganzen den 24stundenrhythmischen Variationen der Gallenmenge folgten.

*Koster, Sharpiro und Lemer* (1936) registrierten 16 Tage lang die Gallenmenge bei einem Patienten mit Gallenfistel. Sie konstatierten einen markanten 24Stundenrhythmus mit deutlichen, um etwa 9 und 21 Uhr statistisch gesicherten Gallenmaxima. Es wurde ferner festgestellt, dass die gallensekretorische Tätigkeit der Leber keine sichere Steigerung infolge von Nahrungszufuhr erkennen liess.

*Forsgrens* Entdeckung der 24stundenrhythmischen Tätigkeit der Leber ist von zahlreichen Untersuchern bestätigt worden. *Hjalmar Holmgren* (1936) und *Holmqvist* (1931) verwendeten als Versuchstiere Ratten, *Ågren, Wilander und Jorpes* (1931), *Holmgren* (1931) Mäuse. *U. v. Euler und Holmqvist, Sjögren, Nordenskjöld, Holmgren und Möllerström* hatten wie *Forsgren* Kaninchen als Versuchstiere, *Petrén* (1939) Meer-schweinchen.

Der Zeitpunkt der diesbezüglichen Maxima und Minima wechselt bei den verschiedenen Tierarten, und das Nachtmaximum ist in gewissen Fällen unsicher. Sämtliche Untersucher haben eine relative Unabhängigkeit von der Nahrungsaufnahme gefunden.

Auch *Higgins, Berkson und Flock* (1932) haben an Ratten 24stundenrhythmische Variationen im Glykogen-, Wasser-, Protein- und Fettgehalt der Leber nachgewiesen. Diese Autoren wollen jedoch diesen Rhythmus auf alimentäre Einflüsse zurückführen. Trotz einer irreführenden Versuchsanordnung geht aus den eigenen Versuchsergebnissen derselben deutlich hervor, dass die Nahrungsaufnahme allein nicht für die Glykogenmenge bei ihren Versuchen bestimmend war, sondern dass auch endogene Faktoren im Spiele waren.

Die normalen Variationen des Glykogensgehalts der Leber gehen mit Schwankungen der Weite der Leberkapillaren Hand in Hand. *Holmgren, Engström und Wohlfart* (1936) haben gezeigt, dass die Blutkapillaren der Leber während des glykogenarmen Stadiums bis fünfmal so viel Blut enthalten können wie im glykogenreichen Assimilationsstadium. Es ist eine un-

gelöste Frage, ob es sich um ein primär vasomotorisches Phänomen mit zentralen Nervenimpulsen handelt, welches die alternierende Glykogenspeicherung und Gallenbildung der normalen rhythmischen Leberfunktion regelt, oder ob es irgendein anderer den Impuls gebender Faktor ist, der bei der Glykogensynthese wirksam ist.

*Sjögren, Nordenskiöld, Holmgren und Möllerström* (1938), welche in Versuchen an Kaninchen die *Forsgrenschen* Befunde bestätigen konnten, haben wie dieser gefunden, dass das Leberglykogen des Kaninchens bei Nahrungszufuhr in 24 Stunden mit zwei Maxima variiert (Abb. 3). Das erste Maximum tritt am Morgen, etwa um 5 Uhr, auf, das zweite am Nachmittag, ungefähr um 6 Uhr. Die Glykogenminima fallen in diesem Versuch auf etwa 3 Uhr und ca. 9 Uhr. Futter wurde bei dem Versuch um 7 Uhr und 16.30 Uhr gegeben.

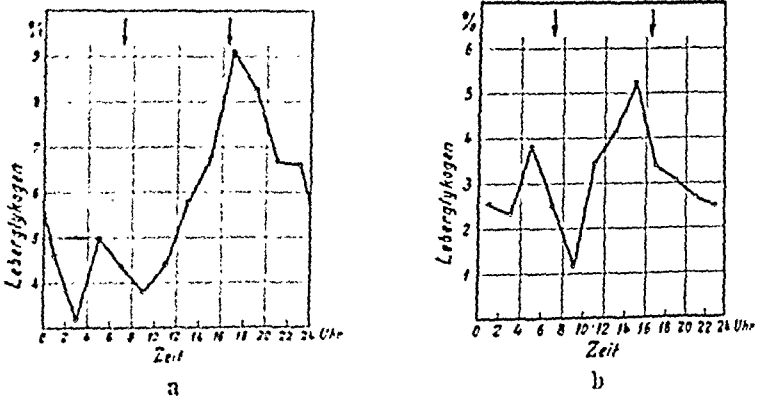


Abb. 3. Glykogengehalt der Kaninchenleber während der 24-Stundenperiode bei Nahrungszufuhr. a Männchen, b Weibchen. Die Pfeile markieren den Zeitpunkt der Fütterung (nach *Sjögren, Nordenskiöld, Holmgren und Möllerström*).

Bei hungernden Tieren verschwindet das erste Glykogenmaximum; das zweite dagegen bleibt noch nach 48stündigem Hunger unverändert bestehen, mit einem Minimum etwa um 13 Uhr (vgl. Abb. 4).

Bei längerer Nahrungsentziehung, 48 Stunden bei Weibchen und 72 bei Männchen, verhält sich der Glykogengehalt der Leber unregelmässiger, was wahrscheinlich auf eine Störung in der normalen Funktion der Leberzellen hindeutet.

*Diese Untersuchungen lehren, dass beim Kaninchen das klei-*

nere Glykogenmaximum (das Nachtmaximum) alimentären Ursprungs ist, das grössere Maximum dagegen (das Tagesmaximum) endogenen Charakter hat.

Auffallend ist der höhere Leberglykogengehalt der Männchen im Vergleich zu dem der Weibchen. Noch nach 48stündigem Hungern enthalten die Lebern der Männchen im Assimilationsstadium mehr Glykogen als die der Weibchen nach nur 24stündigem Fasten (vgl. Abb. 4).<sup>1</sup>

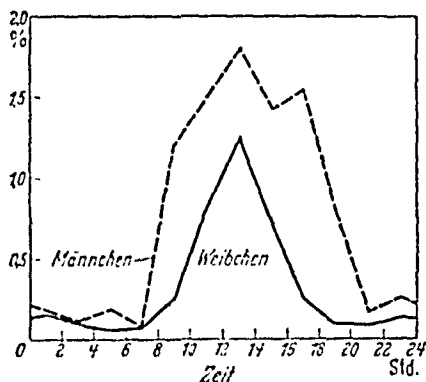


Abb. 4. Glykogengehalt der Kaninchenleber während der 24 Stundenperiode bei Nahrungskarenz. Weibchen nach 24stündigem, Männchen nach 48stündigem Hungern (nach Sjögren, Nordenskiöld, Holmgren und Möllerström).

Die grössere Leberglykogenkapazität der Männchen gegenüber den Weibchen war schon 1930 von Bockelmann und Dieckmann nachgewiesen worden. Die Beobachtungen dieser Autoren sind durch die Untersuchungen von Sjögren, Nordenskiöld, Holmgren und Möllerström in allen Punkten bestätigt worden.

Bei Studien über die Menge des Leberglykogens ist es daher erforderlich, das Geschlecht der Versuchstiere zu berücksichtigen. Offenbar besteht ein merkwürdiger physiologischer Geschlechtsunterschied sowohl in bezug auf die Glykogenbildung der Leber als auch hinsichtlich der Glykogenkapazität derselben, Faktoren, welche von grundlegender Bedeutung für den Energiehaushalt des Organismus sind (vgl. S. 42).

Bockelmann, Dieckmann, Kaufman und Schering zeigten 1931, dass die Glykogenmenge in der Leber in Beziehung zum Brunstzyklus steht. Butts und Deuel haben 1933 angegeben,

<sup>1</sup> Nachtrag bei der Korrektur:

Bomskow und Kaulla haben bei kürzlich veröffentlichten Untersuchungen (Zschr. f. d. ges. exp. Med. 110, 603, 1912) unter Beachtung gewisser äusserer Versuchsbedingungen keinen Wechsel im Glykogengehalt der Leber gefunden. Sie bestreiten daher das Vorliegen einer 24stundenperiodischen Leberglykogenbildung. Bei den Versuchstieren wurde der Glykogengehalt nur an zwei verschiedenen Zeitpunkten ermittelt, welche unglücklicherweise so gewählt sind, dass die Glykogenmengen an den beiden Zeitpunkten dieselben sind, aber das eine Mal ist die Glykogenkurve im Steigen, das andere im Sinken, weshalb diesen Versuchen keinerlei Beweiskraft beigemessen werden kann.



dass ein bestimmter Unterschied zwischen den Geschlechtern in bezug auf den Kohlehydratstoffwechsel vorliegt, und dass Frauen gegen Hunger empfindlicher sind als Männer.

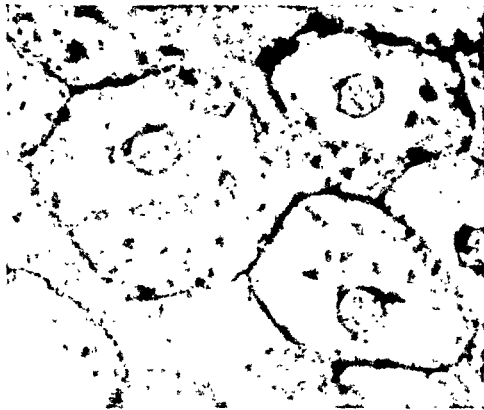
## Resorption und weitere Schicksale des Nahrungsfetts

Das mit der Nahrung aufgenommene Fett wird bei der Verdauung durch die Wirkung der Pankreaslipase in Glycerin und Fettsäuren gespalten.

Bei der Fettresorption spielen nach *Vérzar* die Gallensäuren und wahrscheinlich auch das Nebennierenrindenhormon eine wichtige Rolle. Bei der Spaltung des Neutralfetts während der Verdauung in Glycerin und Fettsäuren wird das wasserlösliche Glycerin direkt von den Darmepithelzellen resorbiert. Die Fettsäuremoleküle dagegen gehen eine komplexe Verbindung mit den hydrotrop wirkenden Gallensäuren ein. Diese komplexe Gallensäure-Fettsäureverbindung kann von den Epithelzellen aufgenommen werden. Infolge ihrer starken Oberflächenspannung wandern die Gallensäuren durch die Zelloberfläche zurück und können dann neue komplexe Verbindungen mit freien Fettsäuren im Darminhalt bilden. Dieselbe Gallensäuremenge kann mithin grosse Mengen von freien Fettsäuren in das Darmepithel hineinbefördern.

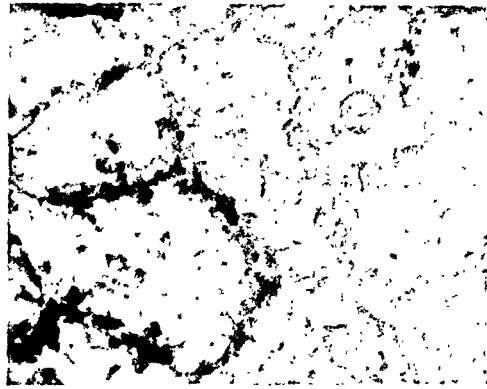
Die in den Darmepithelzellen von neuem freigemachten Fettsäuren verbinden sich mit dem resorbierten wasserlöslichen Glycerin zu Neutralfett, welches also in der Darmwand wieder entsteht. Man findet dasselbe in Form von Fetttröpfchen bereits in den Epithelzellen (Abb. 6). Dieses Darmfett wandert weiter zum zentralen Chylusraum der Darmzotten, von dort auf dem Wege über die Lymphbahnen zum Ductus thoracicus und ergiesst sich mit dem Chylus in den Angulus venosus. Das resorbierte Nahrungsfett passiert daher nicht in nennenswertem Ausmass die Leber und das Pfortadersystem. Es geht in erster Linie durch das Blutkapillarensystem der Lungen.

Die Rolle der Lungen beim Fettstoffwechsel ist noch nicht völlig geklärt. Schon *Boerhave* (1740), *Haller* (1788) und *As-*



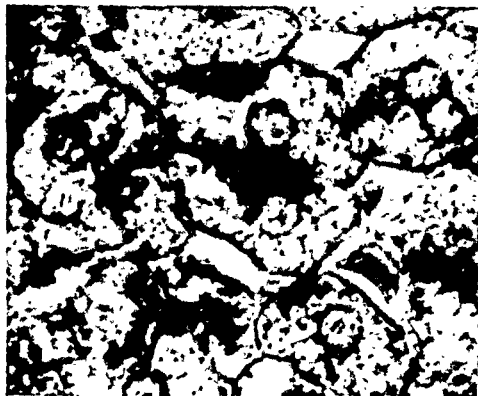
a. Höhepunkt der assimilatorischen Phase.

Versuchstier 80, getötet um 0.15 Uhr. Glykogengehalt der Leber 11 %. Leberzellen sehr voluminös, Zytoplasma zeigt netzförmige Struktur, mit kleinen Eiweißklumpen in den Knotenpunkten des Netzes, aber nur einzelne Sekretgranula. Die Gallenkapillaren sind leer und zusammengefallen.



b. Beginnende Sekretion.

Versuchstier 82, getötet um 4 Uhr. Glykogengehalt der Leber 11 %. Sekretgranula haben sich längs der Gallenkapillaren angesammelt, die jedoch immer noch leer und fast zusammengefallen sind.



c. Fortgeschrittene Sekretion.

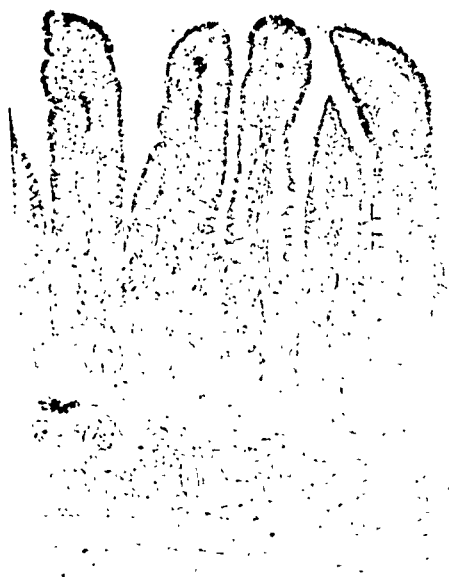
Versuchstier 83, getötet um 6 Uhr. Glykogengehalt der Leber 5.2 %. Zwischen Kern und Gallenkapillare ist in den Leberzellen eine »sekretorische Zone« mit zahlreichen Sekretgranula zu beobachten. Gallenkapillaren weit und mit Galle gefüllt.



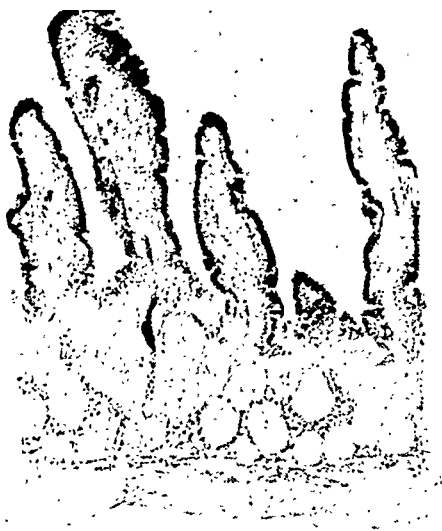
d. Höhepunkt der sekretorischen Phase.

Versuchstier 60, getötet um 11.30 Uhr. Glykogengehalt der Leber 1 %. Die Leberzellen sind klein und enthalten reichlich Sekretgranula. Gallenkapillaren maximal weit, mit Sekret gefüllt.

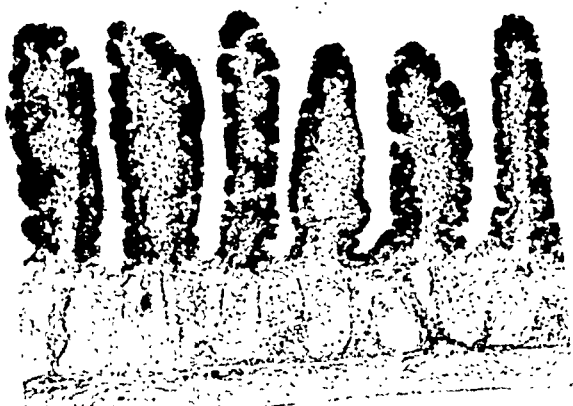
Abb. 5. Die rhythmische Leberfunktion. Abwechselnd Glykogenbildung und Gallensekretion, dem assimilatorischen bzw. dissimilatorischen Funktionsstadium entsprechend (nach Forsgren).



a



b



c



d



e

Abb. 6. Verschiedene Phasen der Fettresorption in den Darmzotten der Ratte bei fettreicher Kost. Darminhalt während der ganzen Zeit fetthaltig.

a Beginnende Fettresorption in den Spitzen der Zotten. In Chylusgefäßen der Mucosa Reste von der vorigen Fettresorptionsphase.

b Gesteigerte Fettresorption an der ganzen Zottenoberfläche.

c Maximale Fettresorption. Darmepithel voll, Zotten erigiert.

d Aufhörende Fettresorption. Das Fett verschwindet aus dem Darmepithel und ist in den Chylusgefäßen der Mucosa zu finden. Im Epithel vakuolisierte Zellen.

e Darmzotten im Ruhestadium ohne Fettresorption. Reste resorbierten Fetts sind in basalen Teilen der Zotten zurückgeblieben (nach Hjalmar Holmgren).

*salini* (1792) hatten angenommen, dass die Lungen irgendwie im Dienste des Fettumsatzes stünden, und dass der Chylus in den Lungen in Blut umgewandelt würde.

Während langer Zeit waren indessen die Lungen als Organ für den Fettstoffwechsel in Vergessenheit geraten. Durch Arbeiten von *Roget* und *Binet* (1921—33) wurde die Aufmerksamkeit von neuem auf die Bedeutung derselben für die Verarbeitung des Fetts im Organismus gelenkt. Durch vergleichende Analysen von Blut aus der rechten und linken Herzkammer bei Fettzufuhr unter verschiedenen Versuchsbedingungen und bei direkten Durchströmungsversuchen fanden *Roget* und *Binet*, dass die Lungen einerseits die Fähigkeit besitzen, Fett zurückzuhalten, und andererseits auch imstande sind, Fett abzubauen. Die Arbeiten dieser Forscher sind von einer Reihe französischer Autoren bestätigt worden.

Andere Untersucher dagegen konnten keine derartigen Funktionen des Lungengewebes nachweisen und bestreiten die Bedeutung der Lungen für den Fettstoffwechsel (*Kurivagawa* u.a.).

*Trotcanu* (1930) machte geltend, dass die Fettfixation der Lungen keine dauernde sondern eine vorübergehende sei, was die verschiedenen Untersuchungsergebnisse erklären könnte. Nach *Aschoff* (1926) sind die Lungen gleichwohl als ein im Dienste des Fettumsatzes stehendes Organ zu betrachten.

*Roger* und *Binet* hatten gefunden, dass das Lungenfett einmal in den Lungenkapillaren auftrat, deren Endothelzellen eine aktive Phagozytose intravasalen Fetts erkennen liessen, sodann als freie Fettröpfchen in den Alveolarseidewänden und schliesslich noch in fetthaltigen Zellen im Lungenparenchym, den sog. Alveolarphagozyten, welche entweder von dem Alveolarepithel oder von Mesenchymzellen abstammen.

*Quensel* stellte fest, dass in gewissen Zellelementen des Expectorals, den sog. »grossen Sputumzellen«, sowohl normalerweise wie auch bei Lungentuberkulose Fett- und Lipoideinschlüsse vorhanden sind. In einer untersuchten Sputumprobe fand sich nach *Blix* 1,5 % phosphatidgebundener Phosphor.

*Dragoin* (1929) wies darauf hin, dass die Lungen gesunder Tiere nur geringe Fettmengen enthalten. Das bei diesen zu findende Fett tritt im Alveolarepithel auf.

Wenn das Pankreas exstirpiert worden ist, erscheint dagegen Fett in reichlicher Menge in den Lungenkapillaren, während Endothel- und Alveolarzellen da fettfrei sind. Beim Pankreasdiabetes kommt es daher wahrscheinlich zu Störungen der normalen Beteiligung des Lungenparenchyms am Fettstoffwechsel.

Es ist möglich, dass die Oxydation eines Teils der Fettsäuren im Lungenparenchym stattfindet. Schon a priori ist anzunehmen, dass der reichlich zur Verfügung stehende Sauerstoff sowie die ausgedehnte Oberfläche der Lungenalveolen von grosser Bedeutung für eine intensivere Oxydation sein müssen, welche zur weiteren Verarbeitung der Fettsäuren während des Intermediärstoffwechsels notwendig ist. Die Lungen dienen in diesem Falle nicht nur dem respiratorischen Gaswechsel, sondern auch dem intermediären Fettumsatz beim oxydativen Abbau des Fetts.

Die Resorption des Fetts im Darmepithel ist nach *Holmgren* eine periodische Erscheinung. Das Vorkommen und Auftreten des Fetts im Darmepithel und den Darmzotten geht aus Abb. 6 hervor.

*Hjalmar Holmgren* (1936) hat bei histochemischen Untersuchungen über den Fettgehalt und die Fettverteilung in der Darmwand, dem Lungengewebe und dem Leberparenchym bei Ratten nach Fütterung mit fettreicher Kost eine 24stundenperiodische Variation auch des Fettgehalts gefunden. In einer sich auf 204 gleichgrosse weisse Ratten desselben Geschlechts erstreckenden Versuchsreihe fand *Holmgren* folgendes:

Das *Darmfett* sank in den Morgen- und Vormittagsstunden und erreichte um 14 Uhr ein Minimum. Von da an stieg der Fettgehalt des Darms, war um 2 Uhr bei seinem höchsten Wert angelangt und begann dann wieder zu fallen;

das *Lungenfett* wies ein Minimum um 16 Uhr und ein statistisch sicheres Maximum zwei Stunden später, um 18 Uhr, auf;

das *Leberfett* zeigte ein deutliches Minimum um 14 Uhr, ein statistisch wahrscheinliches Maximum um 2 Uhr;

die 24Stundenvariationen des *Leberglykogens* traten bei denselben Tieren mit einem Minimum um 20 Uhr und einem Maximum um 8 Uhr in Erscheinung.

Jegliche Nahrungsaufnahme wurde bei diesen Versuchen re-

gistriert. Trotz freien Zugangs zum Futter frassen die Tiere hauptsächlich am Abend und in der Nacht, von etwa 17 Uhr bis 6 Uhr, und zwar ziemlich kontinuierlich. An den übrigen Zeiten der 24Stundenperiode frassen die Tiere nur wenig und mehr sporadisch. Die Ratten sind ja dafür bekannt, dass sie ihre grösste Aktivität nachts entwickeln.

Im Magen-Darmlumen befand sich bei den Versuchstieren während der ganzen 24Stundenperiode reichlich Nahrung und Fett, weshalb die Variationen im Fettgehalt nicht durch Nahrungsmangel bedingt sein können.

Die 24Stundenvariationen des Darm-, Lungen- und Leberfetts sowie des Leberglykogens veranschaulicht Abb. 7 (nach Holmgren).

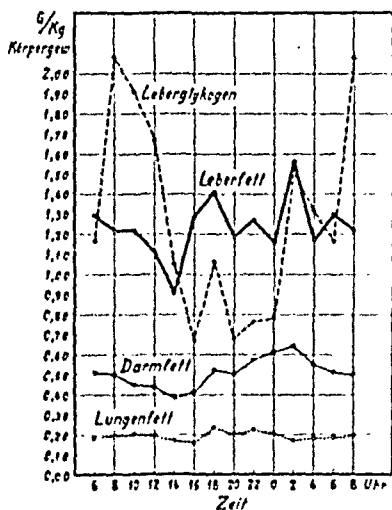


Abb. 7. Variationen des Darm-, Leber- und Lungenfetts während der 24Stundenperiode bei fettreicher Kost (Ratte) sowie entsprechende Veränderungen im Glykogengehalt der Leber (nach Hjalmar Holmgren).

### Die Bedeutung des Fettgewebes für den Kohlehydratstoffwechsel

Ein Überschuss von Kohlehydraten in der Nahrung bewirkt nicht nur eine Glykogenzunahme, sondern auch eine Vermehrung des Fettvorrats im Organismus. Auf der anderen Seite kann Fett bei mangelhafter Kohlehydratzufuhr normalerweise in Zucker umgewandelt werden und auch als Glykogenquelle dienen. Fett und Kohlehydrate können somit ineinander übergehen, und bei dieser Transformation spielen auch die Leber und das Fettgewebe eine wichtige Rolle.

Bei Versuchen an Ratten haben Schur und Löw eine Zunahme des Zuckergehalts im Netz nach kohlehydratreicher Kost gefunden. Diese Autoren betrachten das Netz und das abdominale Fettgewebe als eine Durchgangsstation für resor-

bierte Kohlehydrate. Bei Kohlehydratüberschuss in der Nahrung wird der Zucker im Fettgewebe in Neutralfett umgewandelt, das im Netz als Depotfett gespeichert wird.

Schur und Löw haben ferner beobachtet, dass der Zuckergehalt des Netzes auch bei anhaltendem Hungern steigt. Dies deutet auf eine Kohlehydratbildung im Fettgewebe mit Übergang von Fett in Zucker hin, wenn keine Kohlehydrate mit der Nahrung zugeführt werden. Das Fettgewebe ist daher ein aktives Gewebe, welches sich am Kohlehydratstoffwechsel beteiligt und Kohlehydrate zu Depotfett oder Fett zu Zucker umformt, je nach dem Kohlehydratzugang in der Kost. Die stabile Reservennahrung wird im Fettgewebe als Depotfett gespeichert. Zwischen Depotfett und Organfett besteht ein grundsätzlicher Unterschied.

*Depotfett* besteht vorwiegend aus Neutralfett, ist seiner Zusammensetzung nach unspezifisch und wechselt mit der eingenommenen Nahrung. Bei reichlicher Zufuhr körperfremden Fetts mit charakteristischer Zusammensetzung wird dieses im Fettgewebe abgelagert und kann dort mittels seiner charakteristischen Eigenschaften nachgewiesen werden.

Das *Organfett* ist strikt spezifisch, von konstanter Zusammensetzung, weniger gesättigt und ausserdem an Phosphorsäure und Stickstoffbasen zu organspezifischen Lipoiden mit dem Charakter der Phosphatide gekoppelt. Die Phosphatide bilden wahrscheinlich ein wichtiges Zwischenglied bei der normalen Umwandlung des Nahrungs- und Depotfetts im Stoffwechsel (vgl. S. 296).

Burn und Marks (1926) konstatierten bei Perfusionsversuchen an Lebern von Tieren, welche vorher mit reichlichen Fettmengen gefüttert worden waren, dass eine Zuckerbildung vor sich ging, welche sich nicht lediglich durch Umwandlung von Glykogen oder Milchsäure erklären liess. Sie nahmen an, dass es sich um eine Zuckerbildung aus Organfett der Leber handelte.

Jost fand 1931 bei Durchströmungsversuchen an Lebern von phloridzindiabetischen Hunden, dass Phosphatide eine erhebliche Steigerung der Zuckerbildung bewirkten. Gleichzeitig sank der respiratorische Quotient, und der Sauerstoffverbrauch





Der Chylomikrongehalt des Blutes hängt von der mit der Nahrung zugeführten Fettmenge ab. Im Hunger ist der Chylomikrongehalt auch bei schweren Diabetesfällen mit hochgradiger Ketonurie gering (vgl. Tab. 1).

TAB. 1.

Chylomikrongehalt des Blutes während eines Hungertags bei 2 Fällen von bedrohlichem Diabetes. Insulin bei beginnendem Praekoma eingesetzt (nach Bohm, Gernandt, Holmgren und Möllerström):

	B l u t		U r i n	
	Zucker- gehalt n. Hagedorn  mg % niedrigst    höchst	Chylomikron- gehalt mittlerer Wert pro Flächen- einheit 8—18 Uhr	Während der Zeit 8—18 Uhr ausgeschiedene Menge	
			Zucker g	$\beta$ -Oxybutter- säure mg
Fall 1. 40 I. E. um 16 Uhr	195 — 254	$1,3 \pm 1,0$	11,2	2 799
Fall 2. 40 I. E. um 19.30 Uhr	219 — 270	$2,1 \pm 1,2$	7,6	4 880

Der erhöhte Chylomikrongehalt bei gewissen Fällen von Diabetes hängt womöglich mit einer Störung beim normalen Eingreifen des Lungenparenchyms in den Fettstoffwechsel zusammen. Die Blutgefäße der Lungen bilden das erste Kapillarnetz, welches das resorbierte Fett zu passieren hat, nachdem die Chylusflüssigkeit in die V. subclavia sin. entleert ist. Normalerweise wird indessen das Fett hauptsächlich in den sog. Alveolarphagozyten (vgl. S. 27) angetroffen, in den Lungenkapillaren aber nur in geringerer Menge. Beim Pankreasdiabetes dagegen scheint es in den Alveolarphagozyten nur in kleinen Mengen vorzukommen oder zu fehlen, während es da in grösserer Menge in den Haargefäßen der Lungen auftritt. Zwischen Lipämie und Ketonurie herrscht kein Parallelismus. Bei Fettzufuhr steigt die Anzahl der Chylomikrone, aber un-

abhängig hiervon kann die Ketonurie zu- oder abnehmen, in verschiedenen Fällen wechselnd (vgl. die folgende Zusammenstellung von fünf Diabetesfällen mit Ketonurie, s. Tab. 2).

TAB. 2.

Chylomikrongehalt des Blutes im Hunger und bei fettreicher Kost bei 5 Fällen von Diabetes ohne Insulin.

	B l u t		U r i n	
	Zucker- gehalt n. Hagedorn	Chylomikron- gehalt mittlerer Wert pro Flächen- einheit 8—18 Uhr	Während der Zeit 8—18 Uhr ausgeschiedene Menge	
	mg % niedrigst    höchst		Zucker g	$\beta$ -Oxybutter- säure mg
<b>Fall 1.</b>				
Hunger .....	136 — 247	$2,5 \pm 1,3$	1,2	140
Fettkost.....	129 — 254	$7,8 \pm 5,0$	0	45
<b>Fall 2.</b>				
Hunger .....	172 — 228	$4,9 \pm 2,7$	5,4	545
Fettkost.....	184 — 218	$43,3 \pm 33,5$	8,7	400
<b>Fall 3.</b>				
Hunger .....	75 — 144	$1,3 \pm 0,5$	0	799
Fettkost.....	75 — 117	$13,8 \pm 11,4$	0	95
<b>Fall 4.</b>				
Hunger .....	174 — 241	$1,5 \pm 0,8$	4,5	404
Fettkost.....	172 — 216	$14,4 \pm 12,1$	16,0	1 024
<b>Fall 5.</b>				
Hunger .....	159 — 262	$2,4 \pm 0,3$	2,5	471
Fettkost.....	245 — 298	$31,5 \pm 31,0$	26,2	750

Beim ketonkörperbildenden Diabetes verschwindet die Ketonurie unter dem Einfluss von Insulin. Dabei lässt der Chylomikrongehalt des Blutes eine Zunahme erkennen (vgl. die folgende Tab. 3).

Die Lipämie folgt nicht den Variationen des Blutzuckergehalts und geht nicht der Zuckerdiurese oder Ketonkörperausscheidung parallel (s. folgende Tabelle 4).

TAB. 3.

Einfluss des Insulins auf die  $\beta$ -Oxybuttersäureausscheidung und den Chylomikrongehalt des Blutes bei 4 Fällen von Diabetes.

	B l u t		U r i n	
	Zucker- gehalt	Chylomikron- gehalt	Während der Zeit 8-18 Uhr ausgeschie- dene Menge	
	n. Hagedorn mg % niedrigst    höchst	mittlerer Wert pro Flächen- einheit 8-18 Uhr	Zucker g	$\beta$ -Oxy- butter- säure mg
<b>Fall 1.</b>				
Hunger .....	205 — 240	$2,7 \pm 0,7$	9,7	3 420
Fettkost .....	244 — 289	$42,9 \pm 41,4$	18,4	1 859
Kohlehydratkost .....	263 — 449	$5,1 \pm 2,7$	69,5	1 157
gewöhnl. Kost + Insulin 24 + 24 I. E. ....	287 — 395	$25,6 \pm 17,9$	41,6	0
<b>Fall 2.</b>				
Hunger .....	165 — 218	$2,0 \pm 1,2$	5,3	764
Fettkost .....	197 — 273	$8,9 \pm 8,6$	27,9	829
gewöhnl. Kost + Insulin 32 + 36 I. E. ....	273 — 348	$15,7 \pm 11,4$	76,6	0
<b>Fall 3.</b>				
Hunger .....	73 — 205	$2,0 \pm 1,0$	0,1	319
Fettkost .....	191 — 235	$27,2 \pm 24,4$	13,9	554
gewöhnl. Kost + Insulin 32 + 32 I. E. ....	244 — 325	$27,1 \pm 21,9$	54,9	0
<b>Fall 4.</b>				
Hunger .....	184 — 357	$1,5 \pm 0,6$	3,5	83
Fettkost .....	224 — 258	$14,7 \pm 7,7$	3,9	237
gewöhnl. Kost + Insulin 20 + 20 I. E. ....	214 — 432	$20,0 \pm 29,8$	63,0	0

Der Chylomikrongehalt des Blutes nach Fettzufuhr zeigt mehr oder minder starke 24Stundenvariationen (Abb. 8).

TAB. 4.

Zucker- und Chylomikrongehalt des Blutes sowie ausgeschiedene  $\beta$ -Oxybuttersäuremenge bei Diabetes mit verschiedenem Nahrungsregime.

H u n g e r			F e t t k o s t			K o h l e h y d r a t k o s t		
Blut- zucker mg %	Chylo- mikrone	$\beta$ -Oxybut- tersäure- ausschei- dung 8-20 Uhr mg	Blut- zucker mg %	Chylo- mikrone	$\beta$ -Oxybut- tersäure- ausschei- dung 8-20 Uhr mg	Blut- zucker mg %	Chylo- mikrone	$\beta$ -Oxybut- tersäure- ausschei- dung 8-20 Uhr mg
157-188	$2,2 \pm 0,4$	30	138-184	$41,0 \pm 55,6$	23	231-430	$1,5 \pm 0,2$	40
82-106	$2,0 \pm 1,8$	306	88-265	$7,0 \pm 5,2$	43	280-466	$4,1 \pm 2,9$	552
174-241	$1,5 \pm 0,8$	403	184-218	$43,3 \pm 33,5$	400	249-381	$2,2 \pm 1,2$	840
75-144	$1,3 \pm 0,5$	799	197-273	$8,9 \pm 8,6$	829	254-440	$5,5 \pm 2,8$	935
280-313	$1,5 \pm 0,4$	2 561	172-216	$14,4 \pm 12,1$	1 024	263-449	$5,1 \pm 2,7$	1 157
195-254	$1,3 \pm 1,0$	2 780	218-247	$12,5 \pm 10,9$	1 744			
219-270	$2,1 \pm 1,2$	4 880	244-289	$48,9 \pm 41,4$	1 859			

Die Ketonkörperbildung erfolgt aus den Fettsäuren bei unvollständigem oxydativem Abbau derselben. Bei Fütterungsversuchen hat *Schwartz* gefunden, dass es hauptsächlich nie-

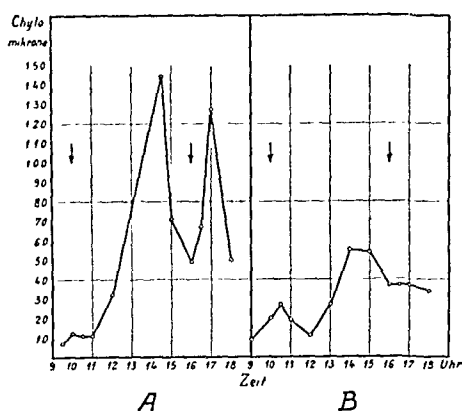


Abb. 8. Variationen des Chylomikrongehalts im Blutplasma während des Tages bei Nahrungszufuhr ohne Insulin. Die Pfeile bezeichnen die Mahlzeiten.

a Diabetes mit Ketonurie. b Diabetes ohne Ketonurie.

dermolekulare Fettsäuren sind, welche die Azetonkörperausscheidung bei Diabetikern steigern. Die Propionsäure macht jedoch eine Ausnahme und bewirkt keine vermehrte Ketonurie. Daher steigt die Zuckerausscheidung nicht, wenn Fettsäuren beim Diabetes als Nahrung zugeführt werden, wohl aber tritt in gewissen Fällen eine stärkere Ketonurie auf.

Bei Perfusionsversuchen an überlebenden Lebern hat *Embden* gezeigt, dass die Ketonkörperbildung hauptsächlich aus Fettsäuren mit einer geraden Anzahl von Kohlenstoffatomen stattfindet. Die relative Ketonkörperbildung aus verschiedenen Fettsäuren wird aus der folgenden Zusammenstellung nach *Embden* ersichtlich.

Normal-Butter-	Säure mit 4 Kohlenstoffatomen	128
» Valerian-	» » 5	» 20
» Kapron-	» » 6	» 100
» Heptyl-	» » 7	» 12
» Oktyl-	» » 8	» 60
» Nonyl-	» » 9	» 19
» Dekan-	» » 10	» 58

Fettsäuren mit höherem Molekulargewicht, Stearin- und Ölsäure, bewirken bei Fütterungsversuchen nur eine geringfügige Zunahme der Azetonkörperausscheidung.

Auch Eiweiss kann wie Fett für die Zuckerbildung des Organismus in Anspruch genommen werden. Dabei sind gewisse Aminosäuren wie die Fettsäuren ketonkörperbildend, andere dagegen zuckerbildend. Zu den *ketoplastischen* Aminosäuren werden Leuzin, Phenylalanin und Tyrosin gerechnet. In dem Masse, wie ketoplastische Aminosäuren in dem Eiweiss enthalten sind, gibt dieses bei gestörtem Kohlehydratstoffwechsel zur Entstehung von Ketonkörpern Anlass. Über die *glykoplastischen* Aminosäuren vgl. S. 18.

Die Kohlehydratbildung aus Fett findet wahrscheinlich in der Leber im Zusammenhang mit der periodischen Glykogenbildung statt. Bei Störungen in der Umwandlung des Fetts in Zucker tritt eine abnorme Ketonkörperbildung ein. Auch diese folgt der rhythmischen Leberfunktion. Bei gewissen Fällen von Diabetes spiegelt sich daher die rhythmische Lebertätigkeit sehr

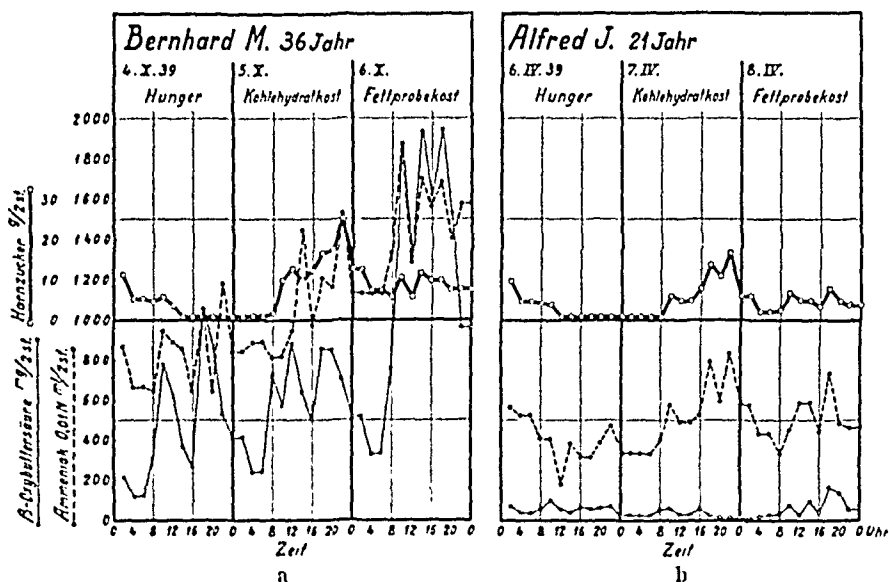


Abb. 9.  $\beta$ -Oxybuttersäureausscheidung im Hunger ohne Insulin bei ketonkörperbildendem Diabetes.

- a Mit stark hervortretender rhythmischer Ketonkörperausscheidung.  
b Ohne periodische Ketonurie.

deutlich in dem periodischen Verlauf der Ketonkörperausscheidung wieder, besonders bei Hunger (Abb. 9). Bei anderen Fällen dagegen tritt eine periodische Ketonurie nicht in entsprechendem Grade hervor. Es ist nicht möglich, zu sagen, ob es sich hierbei in den einzelnen Fällen um einen verschiedenen Ursprung der Ketonkörperbildung handelt, oder ob es irgendwelche anderen Faktoren sind, welche für den Verlauf der Ketosäureausscheidung bestimmend sind (vgl. S. 147 u. 376).

Die Beziehung zwischen Ketonkörperbildung und Zuckerausatz lässt sich bei schrittweiser Herabsetzung der Insulindosis bei Diabetes mit Ketonurie verfolgen. Bei konstanter Kohlehydratzufuhr hält sich da die Zuckerausscheidung verhältnismässig auf derselben Höhe, aber die Ketonkörperausscheidung steigt mit fallender Insulindosis (Abb. 10). Dies spricht dafür, dass das Insulin in die ketonkörperbildenden Prozesse eingreift. Die Insulinwirkung auf die Zuckerausscheidung scheint dagegen ein sekundäres Phänomen zu sein (vgl. S. 275 u. 295).

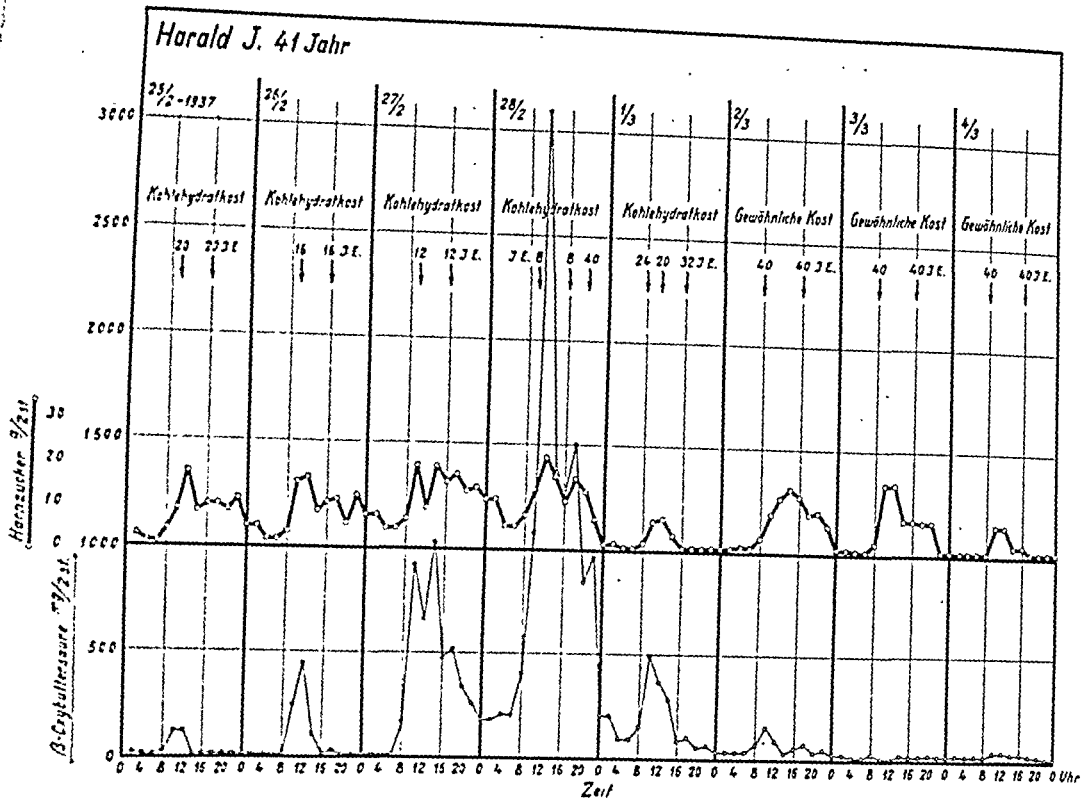


Abb. 10. Bei konstanter Nahrungszufuhr, aber sukzessiver Verminderung der Insulindosis bleibt bei ketonkörperbildendem Diabetes die Zuckerdiurese im wesentlichen unverändert, im Gegensatz zur Ketonurie, welche rasch zunimmt. Wird die Insulindosis gesteigert und den Ketosäurewellen im Ausscheidungsdiagramm richtig angepasst, so verschwindet die Ketonurie auch bei freierer Kost und weiterbestehender Zuckerausscheidung. Ist die Insulindosis gross genug, um den Stoffwechsel in die Gleichgewichtslage zu bringen, so geht die Zuckerdiurese nach und nach zurück, wobei der Insulinbedarf geringer wird.

## Glykogen und Hormone

Zur Bildung des Glykogens ist nicht nur das Material für die Glykogensynthese erforderlich, sondern auch die Mitwirkung von Biokatalysatoren, welche den Assimilationsprozess beherrschen und den Verlauf desselben in die rechte Bahn lenken. Die wirksamen Substanzen sind dabei teils exogenen Ursprungs — die Vitamine —, teils endogenen — die Fermentsysteme und Hormone. Die Wirkung derselben wird im IV. und V. Abschnitt eingehender behandelt werden.

Auch die Mobilisierung des Glykogens ist von der Biokatalysatorwirkung bzw. von hormonalen Einflüssen abhängig. So

wird der Glykogengehalt der Leber von neurohormonalen Impulsen geregelt, welche für die Umsetzung der Kohlehydrate im Organismus bestimmend sind. Hierdurch wird wahrscheinlich auch die periodische Leberfunktion gesteuert.

## Die Hypophyse

Die Hypophyse ist ein Regulator für die Kohlehydratumsatzung im Organismus. Die Wirkung derselben ist von komplexer Natur und wird im VI. Abschnitt im Zusammenhang mit der Frage der neurohormonalen Steuerung des Kohlehydratstoffwechsels näher erörtert werden.

Bei Unterfunktion des Hypophysenvorderlappens in Fällen von hypophysärer Kachexie (*Morbus Simmond*) und hypophysärem Zwergwuchs fand *Cushing* (1922) niedrige Nüchternblutzuckerwerte mit Neigung zu hypoglykämischen Schocken. Nach Entfernung der Hypophyse im Tierversuch stellte *Braier* (1931) fest, dass der Blutzuckergehalt im Hunger auf hypoglykämische Werte sinkt. Nach *White* sterben Kaninchen schon 24 Stunden nach der Hypophysektomie im hypoglykämischen Koma.

*Corkill, Marks* und *White* fanden eine hochgradige Verminderung des Glykogenvorrats der hungernden Tiere nach Hypophysektomie. Der Glykogenzerfall war dabei im Vergleich zu dem normaler hungernder Kontrolltiere gesteigert. Glykoseinjektionen können nach *Cope* (1937) die hungernden hypophysektomierten Tiere temporär am Leben erhalten.

*Houssay* sowie *Russell* und *Bennet* (1936) vermochten durch Injektion eines Extrakts aus Hypophysenvorderlappen oder durch Implantation der Hypophyse das Zustandekommen des hypoglykämischen Komas bei hypophysenlosen Tieren zu verhindern und den Glykogenzerfall zu vermindern.

*Alles dies deutet darauf hin, dass die Hypophyse wirksame Faktoren enthält, welche die Glykogenbildung im Organismus beim Hungern fördern.*

*Soskin, Zimmermann* und *Crohn* (1936) haben gefunden, dass einseitige Fett-nahrung bei hypophysektomierten Tieren



wie Hunger wirkt, indem eine hochgradige Hypoglykämie eintritt und das Glykogen verschwindet. Demgegenüber kann eiweissreiche und auch kohlehydratreiche Kost die Entstehung der Hypoglykämie verhindern. Die Mitwirkung der Hypophyse ist daher für den Übergang des Fetts in Kohlehydrate erforderlich, und diese Transformation geht mit Ketonkörperbildung als Zwischenstufe vonstatten. Bei gestörtem Kohlehydratstoffwechsel löst also die Hypophyse durch die Wirkung des ketogenen Hypophysenhormons, von welchem im VI. Abschnitt eingehender die Rede sein wird, eine Ketonkörperbildung aus.

Zahlreiche Untersuchungen sind über das Verhalten des Leber- und Muskelglykogens nach Hypophysektomie angestellt worden.

Bei reichlicher Nahrungszufuhr scheint die Hypophysektomie den Leber- und Muskelglykogengehalt nicht nennenswert zu beeinflussen. *Chaikoff, Holtom und Reichert* konstatierten bei Versuchen an Hunden normale Leberglykogenwerte. Auch das Muskelglykogen wies da nur unerhebliche Abweichungen von normalen Werten auf. *Cope* (1936) fand bei Versuchen an Kaninchen ebenfalls keine Veränderung des Glykogengehalts der Leber und auch normale Muskelglykogenwerte bei reichlicher Nahrungszufuhr.

Im Hunger stellte dagegen *Cope* bei hypophysektomierten Kaninchen eine starke Abnahme des Leberglykogengehalts sowie gleichzeitig sinkende Blutzuckerwerte fest. Wenn da ein hypoglykämischer Schock eintrat, war die Leber bei den hungernden Tieren nahezu glykogenfrei. Nach *Cope* ist die Hungerhypoglykämie eine Folge des sinkenden Glykogengehalts der Leber.

*Russell und Bennett* (1937) fanden bei Versuchen an Ratten ebenfalls normale Leber- und Muskelglykogenwerte bei den hypophysektomierten Tieren bei reichlicher Nahrungszufuhr. Im Hunger sank dagegen das Glykogen schon nach 8 Stunden sehr stark. Besonders hochgradig war die Abnahme des Leberglykogens.

Die folgende Tabelle 5 nach *Russell* macht die Veränderungen des Glykogen- und Blutzuckergehalts nach Hypophysektomie ersichtlich.

TAB. 5.

Abnahme des Glykogen- und Blutzuckergehalts im Hunger bei normalen und hypophysenlosen Ratten in Prozent des Ausgangswerts des Glykogengehalts bei den wohlgenährten Versuchstieren (nach Russell).

	Nach 8stündigem Fasten		Nach 18stündigem Fasten	
	normale	hypophysenlose	normale	hypophysenlose
Leberglykogen .....	27 %	95 %	96 %	99 %
Muskelglykogen .....	8 %	24 %	6 %	41 %
Blutzucker .....	20 %	49 %	32 %	54 %

Jores (1939) fand bei Versuchen an hypophysektomierten Ratten und Meerschweinchen erhebliche Störungen der periodischen Leberglykogen- und Gallenbildung, aber keinen vollständigen Wegfall der rhythmischen Lebertätigkeit. Ein Glykogenmaximum in der Nacht konnte bei den Versuchstieren auch nach Hypophysektomie nachgewiesen werden.

### Insulinüberempfindlichkeit

Houssay und Magenta konstatierten 1924, dass Tiere, welchen die Hypophyse entfernt worden war, *gegen Insulin abnorm empfindlich* waren und bei kleineren Insulindosen als normale Tiere hypoglykämische Krämpfe bekamen. Es scheint dabei nicht direkt der Glykogengehalt zu sein, welcher für die Insulinhypoglykämie bestimmend ist.

Corkill, Marks und White (1934) haben nämlich bei hypophysenlosen Tieren in gutem Ernährungszustand normale Glykogenwerte in der Muskulatur und der Leber auch bei Insulinhypoglykämie gefunden, im Gegensatz zu dem Verhalten bei der Hungerhypoglykämie, wo der Glykogengehalt bei den hypophysenlosen Tieren stark vermindert war.

Nach Crandall und Cherry (1939) hemmt das Insulin bei den hypophysenlosen Tieren den Übergang des Glykogens in Blutzucker, was dagegen bei Normaltieren nicht der Fall ist. Diese hemmende Wirkung des Insulins auf die Mobilisierung

des Leberglykogens und auf den Übergang desselben in Blutzucker soll die Ursache für die Insulinhypoglykämie und die gesteigerte Insulinempfindlichkeit der hypophysektomierten Tiere sein.

## Die Keimdrüsen

*Burlando* (1903) fand bei trächtigen Kaninchen glykogenreichere Lebern als bei unbefruchteten Tieren. Nach dem Wurf sank der Glykogengehalt auf normale Werte ab. *Bockelmann* und *Dieckmann* (1930), *Deuel* u. a. konnten zeigen, dass Rattenlebern während des Praeöstrus und der Östrusperiode mehr Fett und weniger Glykogen enthielten. Bei weiblichen Ratten steigt der Glykogengehalt nach Kastration und kann ebenso hohe Werte wie bei Männchen erreichen (vgl. S. 24).

Die Veränderungen im Glykogengehalt der Leber werden direkt oder indirekt von den Sexualhormonen beeinflusst, möglicherweise durch Vermittlung der Schilddrüse oder des thyreotropen Hormons der Hypophyse (*Capechi*, *Andersen* u. a.). Die Ansichten über die Wirkung derselben gehen auseinander, da verschiedene Untersucher nicht zu den gleichen Resultaten gelangt sind. *Botella-Llusá*, *de Amilibia* und *Mendizábal* (1935) haben festgestellt, dass Follikulin den Glykogengehalt der Leber zum Sinken, Lutein dagegen zum Steigen bringt. Den Schwankungen des Glykogengehalts entsprechend fanden diese Forscher bei mit Follikulin behandelten Tieren eine Hyperglykämie, bei mit Lutein behandelten aber eine Hypoglykämie. Andere Autoren berichten über nicht eindeutige Wirkungen. Das Hodenhormon löst nach *Deuel* (1933) eine Zunahme des Leberglykogens aus, *Parhon* und *Cahane* (1937) konstatierten hingegen bei Kaninchen und Ratten ein Sinken des Glykogengehalts der Leber.

Die schwankenden Ergebnisse der einzelnen Untersuchungen können auf die normalen Variationen des Glykogengehalts der Leber infolge der rhythmischen Tätigkeit dieses Organs zurückgeführt werden. Unter Berücksichtigung der letzteren haben *Edlund* und *Flyger* (1940) bei Versuchen an weissen Mäu-

sen folgende Wirkungen der Sexualhormone auf das Leberglykogen gefunden:

1. Selbst langdauernde Behandlung mit *Corpus-luteumhormon* bewirkt keine Veränderung;
2. das *Follikelhormon* verursacht ein Sinken, u. U. bis unter die Hälfte der Norm;
3. *Testosteronpropionat* löst keine sichere Veränderung aus.

### Die Nebennieren

Die blutzuckersteigernde Wirkung eines Nebennierenextrakts, welche schon *Blum* nachgewiesen hatte, steht in vollem Einklang mit der Beobachtung von *Porges* (1909) über den abnorm niedrigen Blutzuckergehalt bei der Addison'schen Krankheit. Auch im Tierversuch tritt ein starkes Sinken des Blutzuckergehalts nach Nebennierenexstirpation ein. *Pollak* (1909) fand dagegen, dass mit Adrenalin behandelte hungernde Kaninchen eine ebenso glykogenreiche Leber haben konnten wie nach kohlehydratreicher Nahrung. *Kuriyama* (1918) konnte sogar zeigen, dass hungernde, aber täglich mit Adrenalin behandelte Kaninchen grössere Glykogenmengen in der Leber aufwiesen, als die nicht mit Adrenalin behandelten Kontrolltiere.

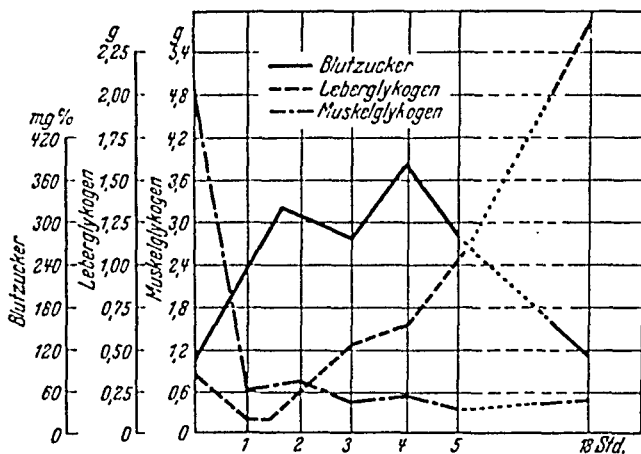


Abb. 11. Veränderungen des Muskel- und Leberglykogens sowie des Blutzuckergehalts nach Adrenalinzufuhr (nach *Cori* und *Cori*).

Schon *Blum* hatte die Beobachtung gemacht, dass Adrenalinzufuhr bei hungernden Hunden nur dann Glykosurie bewirkte, wenn die Tiere fett waren oder vorher mit Fett gefüttert worden waren. Ähnliche Feststellungen sind auch von *Velich* gemacht worden. Dies deutet darauf hin, dass das Adrenalin auch die Neubildung von Zucker aus Fett beschleunigt.

Die vom Adrenalin hervorgerufene Blutzuckersteigerung kann daher nicht auf einer Verminderung des Leberglykogens beruhen. Dagegen nimmt die Menge des Muskelglykogens ab. Abb. 11 zeigt das Verhalten des Blutzuckerspiegels, Muskel- und Leberglykogens während der Adrenalinwirkung (nach *Cori*).

*Cori* hat gezeigt, dass der Milchsäuregehalt des Blutes nach Adrenalinzufuhr rascher steigt als der Blutzuckergehalt. Während der Zeit, in welcher der Blutzuckergehalt zunimmt, ändert sich die Glykogenmenge in der Leber nicht, wohl aber sinkt die der Muskulatur. Wenn dagegen Blutzucker- und Milchsäuregehalt abzunehmen beginnen, dann vermehrt sich nach *Cori* der Glykogengehalt der Leber. Die Glykogenabnahme in der Muskulatur hört da auf (Abb. 12).

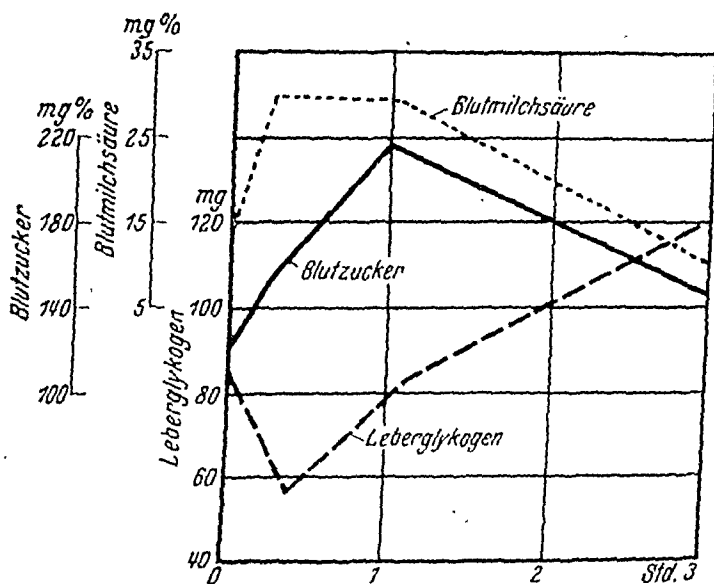


Abb. 12. Blutzucker, Blutmilchsäure und Leberglykogen bei der Adrenalinwirkung (nach *Cori* und *Cori*).

Falls *Coris* Beobachtungen über die Veränderungen des Leber- und Muskelglykogengehalts nach Adrenalinzufuhr nicht auf spontane, mit der periodischen Leberglykogensynthese zusammenhängende Veränderungen zurückzuführen sind, deuten seine Befunde darauf hin, dass das *Adrenalin den anaeroben Abbau des Muskelglykogens beschleunigt und eine gesteigerte Milchsäurebildung bewirkt*.

Die entstandene Milchsäure wird in der Leber in Glykose umgewandelt. Diese Glykose verursacht teils eine Hyperglykämie und Glykosurie, teils eine Speicherung von Leberglykogen. Hierdurch würde unter der Wirkung von Adrenalin der Glykogenvorrat der Muskulatur geleert, aber teilweise in Leberglykogen und Blutzucker übergeführt werden. *Das Adrenalin würde also den Kohlehydratstrom von der Muskulatur zur Leber lenken und dadurch eine im Verhältnis zum Insulin in dieser Hinsicht antagonistische Wirkung erhalten*.

Den Einfluss der Nebennieren auf die Blutzuckerregulation hat *C. G. Sundberg* (1923) an Kaninchen untersucht. Überlebende totalepinephrektomierte Tiere mit oder ohne Rindentransplantat wie auch einseitig nephrektomierte solche konnten eine gänzlich normale Blutzuckerregulation aufweisen. Auch die blutzuckersteigernde Wirkung des Adrenalins war dieselbe wie bei normalen Tieren; dagegen liessen die nebennierenlosen Tiere eine erhöhte Neigung zu hypoglykämischen Zuständen bei Muskelarbeit sowie eine gesteigerte Insulinempfindlichkeit mit hypoglykämischen Symptomen erkennen. Nach Thyroxin- und Adrenalinzufuhr fand *Sundberg* eine anhaltendere posthyperglykämische Blutzuckersenkung als bei normalen Versuchstieren, bei denen eine entsprechende Hypoglykämie nicht eintrat.

Die *Sundbergschen* Befunde wurden von *Lewis* und *Magenta*, *Ballion* und *Gayet* u. a. bestätigt. Auch *Stewart* und *Rogoff* haben die gesteigerte Insulinempfindlichkeit nach Nebennierenentfernung konstatiert. *Barnes*, *Dix* und *Rogoff* (1935) beobachteten, dass diese Insulinempfindlichkeit nach Adrenalinzufuhr abnahm. Die Insulinüberempfindlichkeit tritt sowohl dann ein, wenn die Nebennieren entfernt werden, als auch bei blosser Durchtrennung der Nerven derselben. Die

Autoren waren der Ansicht, die Insulinüberempfindlichkeit, welche auch nach Hypophysektomie auftritt, werde von den Nebennieren vermittelt und beruhe auf mangelnder Adrenalinbildung (vgl. VI. Abschnitt).

Das Adrenalin regt schon in kleinen Mengen durch eine direkt oxydationssteigernde Wirkung in den peripheren Geweben den Grundumsatz stark an.

Über Cortinwirkung vgl. S. 204.

## Die Schilddrüse

*Friedrich v. Müller* stellte 1893 bei der Basedowschen Krankheit einen gesteigerten Sauerstoffverbrauch fest, und in dem gleichen Jahre fand *Mendel*, und auch *Vermehren*, einen erhöhten Eiweisszerfall mit vermehrter Stickstoffausscheidung bei Thyreoidahyperfunktion. *Magnus-Lewy* zeigte 1896, dass der niedrige Grundumsatz beim Myxödem durch Zufuhr von Schilddrüsensubstanz gesteigert wurde.

*Oswald* entdeckte 1896, dass das wirksame Prinzip in der Schilddrüse ein jodhaltiges Thyreoglobulin ist; *Kendall* gelang es 1914, aus demselben durch Hydrolyse in alkalischer Lösung ein kristallinisches Hormon, das *Thyroxin*, rein darzustellen. Dieses besass in grossem Umfang dieselbe biologische Wirkung wie das Thyreoglobulin und die Thyreoidasubstanz (vgl. S. 178).

*Siegel* hat nachgewiesen, dass das Thyroxin den Glykogengehalt der Leber herabdrückt und den postmortalen Glykogenzerfall steigert. Nach Thyreoidektomie ist dies umgekehrt, und der Glykogenzerfall nimmt ab. Bei starker Thyroxinwirkung erfolgt eine beträchtliche Abnahme des Leberglykogens. Auch das Muskelglykogen sinkt, namentlich in der Herzmuskulatur. Der Glykogengehalt der Skelettmuskulatur leidet dagegen weniger.

Thyroxin bewirkt auch eine geringere Fettbildung im Organismus und eine vermehrte Stickstoffausscheidung.

*Eitel*, *Löhr* und *Loeser* haben gezeigt, dass bei erhöhter Schilddrüsenfunktion oder stärkerer Thyroxinwirkung die Ketonkörperbildung zunimmt und der Azetongehalt des Blutes

steigt, und zwar gleichzeitig mit der Verminderung des Glykogens.

*Friedman* und *Gottesmann* haben nachgewiesen, dass die Hyperglykämie und Glykosurie bei pankreasdiabetischen Tieren nach Schilddrüsenexstirpation nachlassen und verschwinden können. Umgekehrt haben *Burn* und *Marks* gefunden, dass Zufuhr von Schilddrüsensubstanz den Blutzuckergehalt steigern kann und bei Diabetikern auch die Glykosurie in die Höhe treibt.

Die vermehrte Verbrennung in den Geweben durch gesteigerte Thyreoideafunktion ruft nach *Houssay* und *Riatti* auch eine erhöhte Empfindlichkeit gegen Sauerstoffmangel hervor (vgl. S. 178 u. 206).

### Die Thymusdrüse

*Bomskow* gab 1939 an, dass er in der Thymusdrüse ein Hormon gefunden hat, welches kräftig in den Kohlehydratstoffwechsel einzugreifen scheint. Das Thymushormon kommt am reichlichsten in den Drüsen junger Tiere vor, weniger bei ausgewachsenen, findet sich aber auch in anderem lymphatischem Gewebe, in der Milz und den Lymphdrüsen, wenn auch in erheblich kleinerer Menge.

Nach *Bomskow* ist das Thymushormon ein Wachstumsfaktor, welcher in der Lipoidfraktion der Thymussubstanz enthalten ist. Es hat Sterincharakter. Die Bildung desselben in der Thymusdrüse wird von dem thymotropen Hypophysenvorderlappenhormon ausgelöst, welches auch einen Diabetes hervorrufen kann (vgl. S. 13).

Das Thymushormon wirkt nach *Bomskow* stark senkend auf den Glykogengehalt der Leber, welcher bei Versuchen an Ratten, Meerschweinchen und Tauben in kurzer Zeit, binnen 6 Stunden, auf einen Bruchteil der normalen Werte fiel. Im Gegensatz zur Wirkung des Thyroxins erfolgt hierbei keine Steigerung des Grundumsatzes oder des Sauerstoffverbrauchs. Das Thymushormon scheint daher nicht wie das Thyroxin in den Energiehaushalt des Organismus einzugreifen.



Das Thymushormon verursacht nach *Bomskow* auch ein Steigen des Blutzuckerspiegels, führt in grösserer Dosis zu einer Zuckerausscheidung und ruft bei gleichzeitigem Insulinmangel einen Diabetes hervor. Andere Autoren (*Rechenberger, Güthert und Schairer, Huf und Ripke, Oberdisse, Hoepke, Anselmino, Albers und Athanasiou* u. a.) haben die *Bomskowschen* Befunde nicht bestätigen können, und die Frage der Existenz des Thymushormons und des thymotropen Hypophysenhormons ist mithin noch ungeklärt (vgl. S. 212 u. 345<sup>1</sup>).

## Glykogen und Vitamine

Die Glykogenbildung ist auch von den mit der Nahrung zugeführten Vitaminsubstanzen abhängig. Es handelt sich dabei um ein Zusammenwirken von endogenen und exogenen Biokatalysatoren, welche einander ergänzen können. Da in dieser Beziehung in erster Linie der Energiehaushalt des Körpers in Betracht kommt, ist das Verhältnis der Vitamine zur Schilddrüse von speziellem Interesse.

Die Vitamine können die Schilddrüse und die Hormone derselben entweder direkt oder indirekt durch Einwirkung auf die Hypophysenfunktion und das thyreotrope Hormon des Hypophysenvorderlappens beeinflussen.

Es ist nicht mit Sicherheit bekannt, ob sich die Vitamine direkt bei der Glykogenbildung im Organismus beteiligen oder ob ihre Wirkung auf dem Wege über das hormonale System vonstatten geht. In gewissen Fällen scheint jedoch die Hypophyse die Wirkung des Vitamins auf den Glykogengehalt zu vermitteln. So verhält es sich mit der Wirkung des Vitamins A. Dieses scheint u. a. die Bildung des thyreotropen Hypophysenvorderlappenhormons zu dämpfen. Wie wir wissen bildet das Vitaminmolekül in anderen Fällen einen integrierenden Bestandteil verschiedener Atmungsfermente; das gilt z. B. für die einzelnen Teile des B-Vitaminkomplexes (vgl. V. Abschnitt).

<sup>1</sup> sowie Nachtrag bei der Korrektur S. 23.

## Vitamin A

*Elmer, Giedosz und Scheps (1935), Fellingner und Hochstädt (1936)* haben gezeigt, dass die thyreotrope Wirkung eines Hypophysenvorderlappenextrakts bei Meerschweinchen geringer wird, wenn gleichzeitig grosse Dosen von Vitamin A zugeführt werden.

*Schulze und Hundhausen (1939)* haben bei Versuchen an Ratten gefunden, dass lange verabreichte A-vitaminfreie Kost eine Zunahme des thyreotropen Hormons der Hypophyse um 30—40 % zur Folge hat. Gleichzeitig wird auch die Schilddrüsenfunktion gesteigert. Reichliche Zufuhr von Vitamin A vermindert die thyreotrope Wirkung der Hypophyse und drückt die Funktion der Schilddrüse herab. *Schulze und Hundhausen* nahmen einen Antagonismus zwischen dem Vitamin A und der thyreotropen Wirkung der Hypophyse an. *Durch diesen Antagonismus wird der Glykogenabbau vom Vitamin A eingeschränkt.*

## Der B-Vitaminkomplex

Dieser ist ein für den normalen Kohlehydratstoffwechsel und die Glykogenbildung wichtiger Faktor. Die physiologisch-chemische Wirkung desselben wird im V. Abschnitt des näheren erörtert werden.

### Vitamin B<sub>1</sub>

Nach *Hundhausen* und *Schulze* nimmt die thyreotrope Wirkung der Hypophyse bei Mangel an Vitamin B<sub>1</sub> in der Nahrung ab. Gleichzeitig lässt auch die Funktion der Schilddrüse nach. Bei B<sub>1</sub>-Mangel wird daher die Thyroxinbildung in der Schilddrüse geringer, was u. a. eine Abnahme des Glykogenzerfalls in der Leber sowie eine Sinusbradykardie zur Folge hat, welche nach *Birch-Harris* durch reichliche Zufuhr von Vitamin B<sub>1</sub>, nach *Parade* auch von Thyroxin, binnen kurzer Zeit behoben werden kann.

*Schneider* hat gefunden, dass auch B<sub>1</sub>-avitaminotische Versuchstiere auf das thyreotrope Hypophysenhormon normal

reagieren. Das Vitamin  $B_1$  scheint somit u. a. die Bildung des thyreotropen Hormons in der Hypophyse anzuregen.

Bei normalen, nicht  $B_1$ -avitaminotischen Tieren mit normaler Bildung von thyreotropem Hypophysenhormon hat nach Untersuchungen von *Gentzen* und *Mohr* das Vitamin  $B_1$  keinen Einfluss auf die Hypophysen- oder Schilddrüsenfunktion.

Widersprechende Angaben über die Bedeutung des Vitamins  $B_1$  für die Glykogenbildung sind zum Teil darauf zurückzuführen, dass die normalen, auf der rhythmischen Leberfunktion beruhenden Schwankungen des Glykogengehalts nicht berücksichtigt wurden.

*Collazo* (1923) fand, dass die Leber bei Hunden, Meerschweinchen und Tauben mit Beriberi trotz Hyperglykämie fast glykogenfrei war. Auch Skelett- und Herzmuskulatur waren nach 20tägiger Fütterung mit  $B$ -vitaminfreier Kost nahezu glykogenfrei. Der Glykogengehalt bei entsprechende Zeit hungrigen Tieren war nach *Collazo* höher als bei den avitaminotischen Tieren.

*Abderhalden* und *Wertheimer* (1932—34) kamen zu dem entgegengesetzten Ergebnis und fanden bei Versuchen an Tauben bei  $B$ -Avitaminose einen erhöhten Glykogengehalt. Eine Reihe Autoren sind zu wechselnden Resultaten gelangt, und es scheinen bei Tauben und Säugetieren verschiedene Verhältnisse vorzuliegen (Schrifttum bei *Edlund* und *Holmgren* [1940]).

Unter Berücksichtigung der periodischen Leberfunktion haben *Edlund* und *Holmgren* (1940) bei Versuchen an Ratten die Frage der Bedeutung des Vitamins  $B_1$  für die Glykogenbildung einer erneuten Prüfung unterworfen. Sie stellten fest, dass:

1. der *Leberglykogengehalt* bei  $B_1$ -Avitaminose sinkt und nach 3 Wochen ein Minimum erreicht,
2. der Glykogengehalt der *Skelettmuskulatur* und anderer Organe während der Avitaminose eine Tendenz zum Steigen aufweist, aber bei lange anhaltendem Vitaminmangel auf normale Werte absinkt. Der Glykogengehalt der *Herzmuskulatur* ist auch bei  $B_1$ -Avitaminose normal und konstant. Der Glykogengehalt des Gehirns zeigt bei  $B_1$ -Avitaminose erhöhte Werte,
3. bei Zufuhr von Vitamin  $B_1$  bei *avitaminotischen* Tieren der Glykogengehalt der Leber steigt, der der Herzmuskulatur

aber unverändert bleibt. In der Skelettmuskulatur und Hirnsubstanz nimmt das Glykogen möglicherweise zu; dies ist jedoch nicht sicher. Wenn der Leberglykogengehalt auf normale Werte gestiegen ist, können auch grosse Dosen von Vitamin B<sub>1</sub> denselben nicht weiter in die Höhe treiben,

4. bei Zufuhr von Vitamin B<sub>1</sub> während der Assimilationsphase der Leber der Leberglykogengehalt nicht beeinflusst wird. Wird dagegen das Vitamin B<sub>1</sub> während der dissimilatorischen Phase der Leberperiode verabfolgt, so scheint der Leberglykogengehalt höher zu sein als bei den Kontrolltieren,

5. auch wenn Glykose gleichzeitig mit Vitamin B<sub>1</sub> gegeben wird, die Glykogenbildung bei normalen Tieren nicht zunimmt.

Das Sinken des Leberglykogengehalts bei B<sub>1</sub>-Avitaminose kann nicht lediglich auf Störungen der Schilddrüsenfunktion beruhen, sondern muss andere Ursachen haben. Möglicherweise ist es der Ausdruck einer Inanition infolge von Anorexie.

### *Vitamin B<sub>2</sub>*

Es ist nicht näher bekannt, ob das Vitamin B<sub>2</sub> eine spezielle Bedeutung für die Bildung und den Abbau des Glykogens im Organismus besitzt. Da das Vitamin B<sub>2</sub> ein Bestandteil gewisser Atmungsfermente ist, muss die Wirkung desselben für die zellulären Lebensfunktionen überhaupt entscheidend sein (vgl. V. Abschnitt).

Nach *Schulze* und *Hundhausen* beeinflusst das Vitamin B<sub>2</sub> die Schilddrüsenfunktion oder das thyreotrope Hormon der Hypophyse nicht.

## Vitamin C

Bei fehlender C-Vitaminwirkung im Organismus treten Störungen des Kohlehydratstoffwechsels ein. Schon *Minkowski* (1885) hatte das Vorkommen von Azeton und  $\beta$ -Oxybuttersäure beim Skorbut nachgewiesen.

*Allenburg* zeigte bei Versuchen an Meerschweinchen, dass der Leberglykogengehalt beim Skorbut herabgesetzt ist. Wird Vitamin C zugeführt, so steigt der Glykogengehalt der Leber.

Wenn man C-avitaminotischen Tieren Glykose gibt, nimmt der Glykogengehalt nicht ohne gleichzeitige Verabreichung von Vitamin C zu. Auch bei normalen Tieren steigt der Leberglykogengehalt, wenn Vitamin C gleichzeitig mit Glykose gegeben wird.

Das Vitamin C scheint nach *Stepp* keinen direkten Einfluss auf den Stoffwechsel auszuüben, greift aber regelnd in die Hormonwirkung im Körper ein und beeinflusst dadurch Glykogenbildung und Kohlehydratumsatz.

*Stepp, Schroeder und Altenburg* (1935) haben nachgewiesen, dass die Ascorbinsäure die Wirkung des Insulins beim Diabetes verstärkt und Azidose und Blutzuckergehalt herabsetzt.

Nach *Altenburg* greift das Vitamin C in den Kohlehydratumsatz der Leber ein und dämpft den durch das Thyreoideahormon gesteigerten Glykogenzerfall. *Schulze und Linnemann* (1938) zeigten, dass das Vitamin C den Gehalt der Hypophyse an thyreotropem Hormon nicht beeinflusst, obwohl beim Skorbut oft Anzeichen einer erhöhten Aktivität der Schilddrüse vorhanden sind. Nach *Teerbrüggen* (1937) und *Sturm, Schmidt und Beck* (1938) mildert dagegen das Vitamin C nicht nur die Wirkung des Thyroxins, sondern auch die des thyreotropen Hypophysenhormons.

*Pfleger und Scholl* (1937) geben an, dass zugeführte Ascorbinsäure den Kohlehydratstoffwechsel des Organismus um so günstiger beeinflusst, je grösser der Mangel desselben an Vitamin C ist. Wenn der Körper mit Vitamin C gesättigt ist, hat die weitere Zufuhr von Ascorbinsäure keine nennenswerte Wirkung.

Da das Vitamin C einen gesteigerten Glykogenzerfall dämpft, wirkt dasselbe beim Diabetes senkend auf den Blutzuckergehalt.

*Söderling und Hamne* (1937) haben Diabetesfälle mit Skorbut beschrieben, bei welchen sich auch der diabetische Zustand nach C-Vitaminzufuhr auffallend besserte.

*Tomaselli* hat bei 11 Fällen von Diabetes nach Verabreichung von Ascorbinsäure ohne Insulin eine konstante Senkung des Blutzuckerspiegels gefunden. Bei Insulinbehandlung wird die Blutzuckersenkung stärker, wenn gleichzeitig Ascorbinsäure gegeben wird.

*Hamne* (1941) hat bei 18 Fällen von Diabetes bei Kindern die alimentäre Hyperglykämie an Hand von 24Stundenkurven mit oder ohne Ascorbinsäure verfolgt. Sämtliche Fälle bekamen Insulin, und die intravenös zugeführte Ascorbinsäuremenge, welche gleichzeitig mit dem Insulin gegeben wurde, variierte zwischen 200 und 500 mg. In sämtlichen Fällen bewirkte die Ascorbinsäure eine Verstärkung der Insulinwirkung, welche sich nach 2 Stunden statistisch sichern liess und bis etwa 6 Stunden nach der Injektion anhielt. Dann liess die Wirkung allmählich nach und war nach ca. 11 Stunden völlig verschwunden.

Bei Glykosebelastungsproben an 32 gesunden Versuchspersonen und Adrenalinproben an 22 solchen fand *Hamne* ebenfalls eine statistisch sichere Verminderung der hyperglykämischen Reaktion bei gleichzeitiger intravenöser Ascorbinsäurezufuhr. Das Vitamin C scheint mithin auch auf den Abbau des Muskelglykogens unter dem Einfluss von Adrenalin dämpfend einzuwirken.

*Eine Vorbedingung für die Besserung eines diabetischen Zustands ist daher, dass die Kost reich an Vitaminsubstanzen ist, welche zum Glykogenaufbau beitragen oder den Zerfall des Glykogens hindern.*

### III. ABSCHNITT

## Leberglykogen und Blutzucker.

Die weite Verbreitung des Glykogens im Organismus mit einer periodischen Neubildung in der Leber sogar im Hunger ist ein deutlicher Hinweis auf die zentrale Bedeutung desselben für den Stoffwechsel. Das Glykogen steht als gespeicherte Substanz im Dienste des Energiehaushalts und lässt bei seinem Abbau niedermolekulare Kohlehydrate entstehen, deren weitere oxydative Aufspaltung die für den Lebensprozess erforderliche Energie liefert.

Der Abbau des Glykogens verläuft in der Leber etwas anders als in der Muskulatur. Aus dem Leberglykogen entsteht als erstes Abbauprodukt eine Hexose, die Glykose, welche wir im Blut als Blutzucker wiederfinden. Das Muskelglykogen dagegen liefert als Resultat eines anaeroben Spaltungsvorgangs eine Triose, die Milchsäure, welche teils oxydativ weiterumgewandelt und teils wieder zu Glykogen resynthetisiert wird. Die biochemischen Reaktionsprozesse werden im IV. Abschnitt eingehender behandelt werden.

Die Bedeutung des Leberglykogens für die Blutzuckerbildung war schon 1903 von *Pavy* und *Siau* nachgewiesen worden. Diese Forscher fanden, dass der Blutzuckergehalt eines Versuchstiers nach Leberexstirpation ständig sank. Binnen kurzer Zeit ging das Tier unter hypoglykämischen Krämpfen zugrunde.

Mit einer verbesserten Versuchsmethodik konnten *Mann* und *Magat* dieses Ergebnis bestätigen. Sie stellten ausserdem fest, dass wiederholte Glykoseinjektionen den Blutzuckergehalt steigerten und bereits moribunde Tiere nach Leberentfernung eine Zeitlang am Leben zu erhalten vermochten. Lävulose konnte in diesem Falle die Glykose teilweise, aber nicht gänz-

lich, ersetzen, Milchsäure und Glykokoll dagegen nicht. Die beiden letzteren Substanzen bewirkten nach Leberexstirpation keine Blutzuckersteigerung, und ebensowenig rief Adrenalin da eine Hyperglykämie hervor. *Mann* und *Magat* fanden ferner, dass die Menge des Muskelglykogens nach Leberexstirpation sinkt, nach Glykoseinjektion aber wieder steigt. Wird eine Anastomose zwischen V. portae und V. hepatica (Ecksehe Fistel) angelegt, so verschwindet im Hunger das Glykogen aus sowohl der Leber wie der Muskulatur. Nur der Herzmuskel behält fast bis zuletzt seinen Glykogenbestand (etwa 0,2 %). Der Blutzuckerspiegel sinkt rasch, und das Blut wird in kurzer Zeit zuckerfrei, wobei das Tier an Hypoglykämie eingeht. Erhält ein Tier mit Eckseher Fistel dagegen Glykose oder leichtresorbierbare Kohlehydrate in reichlicher Menge, so steigt der Blutzuckergehalt eine Zeitlang wieder, und dabei nimmt auch das Muskelglykogen zu. Der Glykogensgehalt der Leber bleibt dagegen nach wie vor niedrig.

Im Hunger muss daher die Leber ständig Zucker an das Blut abgeben, aber bei kohlehydratreicher Nahrung kann der Blutzuckergehalt auch ohne Mitwirkung der Leber beibehalten werden.

*London* (1931) und seine Mitarbeiter haben in Versuchen an Hunden den Zuckerverbrauch verschiedener Organe untersucht. Sie bedienten sich dabei einer Angiostomietechnik, welche die Entnahme von Proben in vivo gestattet. Durch Feststellung der Differenz zwischen dem Zuckergehalt des arteriellen und venösen Blutes der einzelnen Organe lässt sich der Kohlehydratverbrauch unter verschiedenen Bedingungen beobachten. Man fand im Hunger bei Ruhe (Grundumsatz) im Mittel folgende Differenzen zwischen venösem und arteriellem Blut:

Muskeln	7,5 mg %
Gehirn	13,6 "
Ovarien	13,6 "
Därme	9,6 "
Nebennieren	6,6 "
Nieren	5,3 "
Pankreas	5,0 "
Leber	12,0 "



Eine Minusdifferenz bedeutet Glykoseverbrauch im Organ, eine Plusdifferenz dagegen, dass das Organ einen Überschuss an Glykose an das venöse Blut abgibt.

Auch dies macht ersichtlich, dass im Hunger nur die Leber dem Organismus Glykose zur Verfügung stellt, dass alle anderen Organe dagegen Blutzucker in grösserer oder geringerer Menge verbrauchen.

Infolge ihrer grossen Masse, welche sich auf rund 45 % (35—50 %) des Körpergewichts berechnen lässt, ist die Muskulatur das Gewebe, welches bereits in der Ruhe grosse Kohlehydratmengen verbraucht, und wird dadurch zu einem für den intermediären Kohlehydratstoffwechsel sehr wichtigen Faktor.

London und Kotschneff haben ihre Untersuchung auch auf den Unterschied im Blutzuckergehalt bei Nahrungszufuhr mit und ohne Insulin bzw. Adrenalin ausgedehnt. Sie fanden dabei im Mittel folgende Differenzen in mg% zwischen venösem und arteriellem Blut:

	Därme	Leber	Nieren	Muskeln
Hunger .....	— 9	+ 18	— 6	— 5
» + Insulin .....	— 8	+ 17	— 10	— 5
» + Adrenalin .....	— 8,6	+ 23	—	—
Kohlehydratkost .....	+ 29	— 20	— 11	— 12
» + Insulin ....	+ 10	— 9	— 18	— 5
» + Adrenalin ..	0	+ 9	— 10	0
Eiweiss-(Fleisch-) Kost .....	0	0	0	0
» + Insulin .....	0	0	— 14	0
» + Adrenalin .....	0	0	— 10	0
Fett .....	0	0	0	0

*Im Hunger sorgt also die Leber durch Abbau des Leberglykogens für den Zuckerbestand des Blutes. Bei kohlehydratreicher Kost gibt dagegen der Darmkanal einen Überschuss an Glykose an das Blut ab, wobei Zucker auch in der Leber mehr oder weniger energisch zurückgehalten wird.*

Bei den Untersuchungen von London und Kotschneff hat die periodische Leberfunktion keine Berücksichtigung gefunden; dies wäre von grösstem Interesse für die Klärung der Frage der 24stundenrhythmischen Lebertätigkeit gewesen.

## Alimentäre Hyperglykämie

Da der Körper beim Kohlehydratumsatz in den Geweben und Organen Kohlehydrate ununterbrochen verbraucht, muss dem Blut ständig Glykose zugeführt werden, wenn der Zuckerspiegel desselben nicht sinken soll. Dabei erfolgt einerseits eine kontinuierliche, den *Zuckergrundverbrauch* im Hunger deckende Zuckerezufuhr, andererseits eine diskontinuierliche solche gelegentlich der Nahrungszufuhr, welche der *alimentären Zuckeraufnahme* entspricht.

Aus der Nahrung kann nach der Verdauung Glykose direkt im Darmkanal resorbiert werden, dann in das Blut übergehen und dort eine Blutzuckersteigerung bewirken. Im Zusammenhang mit der alimentären Reizung bei der Verdauungstätigkeit kann auch durch den neurohormonalen Regulationsmechanismus eine Mobilisierung von Leberglykogen mit Glykosebildung und Steigerung des Blutzuckergehalts stattfinden. Zahlreiche Untersuchungen sind zwecks Klärung der Frage vorgenommen worden, ob die alimentäre Hyperglykämie eine *Resorptionshyperglykämie* oder eine *Reizungshyperglykämie* ist. Man hat auf verschiedene Weise den Zeitpunkt und die Voraussetzungen für das Auftreten der Hyperglykämie zu bestimmen versucht; den Einfluss des Nervensystems durch pharmakologische oder anatomische Untersuchungen studiert, das Blut vor und nach Passage durch die Leber verglichen, oder im Blut chemisch charakterisierbare Kohlehydrate nach peroraler Zufuhr derselben wiederzufinden gesucht. Die Resultate dieser Untersuchungen waren wechselnd. Zur Erläuterung der Sachlage seien einige Versuchsergebnisse von verschiedenen Forschern angeführt.

*Eisner* und *Forster* fanden eine rasche Steigerung des Blutzuckers schon während der Nahrungsaufnahme. Bei den Versuchen dieser Autoren trat diese Steigerung nur nach kohlehydratreicher Nahrung auf, nicht nach Eiweiss. Sie war von der Menge der zugeführten Kost unabhängig.

*Mahler* und *Rischawy* führten bei gesunden Versuchspersonen mittels einer Duodenalsonde Glykoselösung in verschiedene Abschnitte des oberen Darmkanals ein. Es ergab sich

hierbei, dass schon die Vorbehandlung (das Schlucken der Sonde) binnen wenigen Minuten ein Steigen des Blutzuckerspiegels veranlassen konnte. Der höchste Blutzuckerwert wurde dann erhalten, wenn Glykoselösung in den oberen Teil des Jejunum eingespritzt wurde. Oberhalb und unterhalb dieses Gebiets bewirkte die injizierte Glykoselösung flachere Blutzuckerkurven. Nach Ansicht der Forscher sprechen die Versuchsergebnisse für eine reflektorische Beeinflussung der Leber vom obersten Darmabschnitt aus.

*Klein und Heinemann* untersuchten die Blutzuckerveränderung in der Art. radialis nach intraduodenaler Zufuhr von 25 g Glykose. Sie fanden dabei bei gesunden Versuchspersonen eine Blutzuckerzunahme erst nach 5—6 Min., oft nach kurzem initialem Sinken. Bei Diabetikern und bei einer Versuchsperson mit Gastroenterostomie trat die Blutzuckersteigerung schon nach einer Minute ein. Die Röntgenkontrolle der Mündung der Duodenalsonde ergab in der Regel Lage derselben in der Pars descendens oder horizontalis inferior duodeni (17 Fälle), nur bei dem Gastroenterostomiefall befand sich die Mündung im oberen Teil des Jejunum. *Klein und Heinemann* geben an, dass die Blutzuckersteigerung normalerweise während der ersten Minuten kontinuierlich erfolgt, dass aber die Blutzuckerkurve nach 8—10 Min. einen remittierenden Verlauf mit kurzdauernden Oszillationen annimmt. Bei Diabetikern und bei dem untersuchten Fall von Gastroenterostomie zeigten sich die Oszillationen schon anfangs.

Kurzdauernde Schwingungen im Blutzuckergehalt hatte schon *Karen Marie Hansen* beim Diabetes in sowohl arteriellem wie venösem Blut gefunden. Diese Schwingungen wurden für einen Ausdruck der Wirkung des vegetativ-nervösen Regulationsapparats gehalten. Ob sie eine Manifestation antagonistisch wirkender Kräfte der Blutzuckerregulation sind oder auf einer Ungleichmässigkeit des Blutzuckerstroms aus der Leber beruhen, dürfte nicht leicht zu entscheiden sein. Die Reizungshyperglykämiekurve soll nach *Klein und Heinemann* durch diese Oszillationen ihr charakteristisches Gepräge erhalten; sie sollen bei Resorptionshyperglykämie fehlen. *Klein und Holzer, Albritton* u. a. konnten die Untersuchungsergebnisse von

*K. M. Hansen* bestätigen; *Nielsen* dagegen war nicht imstande, diese kurzdauernden Blutzuckervariationen nachzuweisen. Wahrscheinlich ist das Phänomen nicht konstant. Es ist möglich, dass diese Oszillationen die Ursache dafür bilden, dass zwischen Doppelproben trotz minutiöser Technik bei den Blutzuckerbestimmungen manchmal ein beträchtlicher Unterschied besteht, ein Umstand, auf welchen u. a. *Staub* aufmerksam gemacht hat.

*Chahovitich* und *Arnolcovitch* haben Hyperglykämie nach *intraperitonealen* Injektionen von Bouillon, Pepton und Kaseinlösung nachgewiesen.

*Grunke* hat Ergotoxin verwendet, welches die Nervenendigungen in der Leber lähmen sollte, und dabei gefunden, dass die alimentäre Hyperglykämiecurve nach Glykose einen flacheren Verlauf erhält.

*Hetényi* und *Pogány* haben ebenfalls konstatiert, dass die alimentäre Hyperglykämie nach einer vorher gegebenen Ergotamindosis ausbleibt, und zwar sowohl nach peroraler wie nach intraduodenaler Glykosezufuhr. Nach intravenöser Glykosezufuhr beobachteten diese Autoren dagegen ein langsameres Verschwinden des Blutzuckers. Dies konnte so gedeutet werden, als ob die postalimentäre Hyperglykämie eine durch den Sympathicus ausgelöste Reizungshyperglykämie wäre.

*Pollak* versuchte bei Kaninchen, den Sympathicus durch Ergotamin und den Parasympathicus durch Atropin auszuschalten. Sowohl mit Ergotamin wie mit Atropin vergiftete Tiere wiesen nach peroraler Zuckerzufuhr wechselnde Blutzuckerkurven auf. Die Mehrzahl der Versuche sollte doch eine depressorische Wirkung des Ergotoxins auf die postalimentäre Hyperglykämiecurve demonstrieren. Bei intravenöser Glykosezufuhr wurde diese Beeinflussung nicht beobachtet. Durch Kombination von Ergotamin und Atropin soll sich die postalimentäre Hyperglykämie nach *Pollak* vollständig unterdrücken lassen. Dies beruht seiner Ansicht nach auf einer maximalen Zuckerretention in der Leber. Das vegetative Nervensystem stellt nach *Pollak* einen für die Verteilung der aus der Nahrung stammenden Kohlehydrate zwischen der Leber und peripheren Geweben wichtigen Faktor dar.

*Loeffler* hat gezeigt, dass das Ergotamin auf den vasomotorischen Apparat der Leber einwirkt, und auf die Möglichkeit hingewiesen, dass die nervöse Reizwirkung auf die Leber hierdurch erfolgen könnte.

*Bremer* und *Leclerq* haben Versuche an Hunden und Katzen nach doppelseitiger Durchtrennung der hinteren Wurzeln zwischen Ganglion und Rückenmark, Segment C VII — Th III entsprechend, vorgenommen. Sie kamen nach eingehendem Studium des Reizungseffekts zu der Ansicht, dass es wirklich glykosekretorische Nervenfasern gebe, welche dann durch den N. splanchnicus verlaufen und keine vasomotorischen Veränderungen in den Bauchorganen veranlassen sowie auch keinen Einfluss auf die Herz- oder Atmungstätigkeit ausüben. Durch weitere Untersuchungen an nebennierenlosen Katzen glauben *Bremer* und *Leclerq* erwiesen zu haben, dass zwei Arten von hyperglykämischen Reflexen existieren müssen: einmal zentrale, welche vom N. depressor vermittelt werden und eine Erweiterung der Gefäße des Bauches bewirken, sodann solche, die durch periphere Reizung ausgelöst werden, Segment C VII — Th III durchlaufen und auf dem Wege über den Sympathicus und die glykosekretorischen Fasern desselben vonstatten gehen.

Von der Bedeutung des Nervensystems für die Blutzuckerregulation wird im VI. Abschnitt noch des näheren die Rede sein.

Wenn auch viele dieser Beobachtungen sich in dem Sinne deuten lassen, dass die postalimentäre Hyperglykämie eine Reizungshyperglykämie sein kann, gibt es doch andere, welche weniger leicht durch die Reizungstheorie erklärt werden, vielmehr eine Resorptionshyperglykämie als das Wahrscheinlichere hinstellen.

*Grunke* und *Hesse* haben versucht, bei Kaninchen die Leber glykogenfrei zu machen, so dass Adrenalin keine Hyperglykämie bewirkte, um dann die alimentäre Blutzuckerkurve nach peroraler Glykosezufuhr zu studieren. Die glykogenlose Leber würde in diesem Falle nicht imstande sein, Material für eine Reizungshyperglykämie zur Verfügung zu stellen. Die Glykoselösung wurde mittels Magensonde zugeführt und Kontroll-

versuche mit Wasser vorgenommen. Nach Reduzierung des Glykogenbestandes durch Hunger und Arbeit konnte die Adrenalinhyperglykämie ausbleiben, aber die alimentäre Hyperglykämiekurve wies denselben Verlauf wie bei Normaltieren auf. Die Lebern waren bei den meisten dieser Versuche so glykogenarm, dass Glykogen mit der Pflügerschen Methode nicht mehr nachgewiesen werden konnte. Die Blutzuckerkurve begann trotzdem schon nach 10 Min. zu steigen.

*Gigon* nahm Versuche an mit Äther narkotisierten Hunden vor. Glykose wurde per os gegeben und der Zuckergehalt direkt im Blut aus der V. portae und V. hepatica untersucht. Dabei wurde beobachtet, dass die Leber während der ersten halben Stunde den resorbierten Zucker ohne Retention passieren liess, denn der Blutzuckergehalt war in beiden Gefässen derselbe. Erst später trat eine Blutzuckerdifferenz auf. Nach *Gigon* soll die Glykogenbildung nicht eher in Gang kommen, als der Zuckerbedarf der peripheren Gewebe gedeckt ist. Die alimentäre Hyperglykämie hörte schon auf, bevor der Magen geleert war. Bei Versuchen an Ziegen mit einer Kanüle im Ductus thoracicus fand *Gigon* ferner, dass der Zuckergehalt des Chylus nach Glykosezufuhr mit dem Blutzuckergehalt in der V. portae Hand in Hand ging.

*Katsura* hat bei vergleichenden Untersuchungen keinerlei Beleg dafür erhalten, dass die Kohlehydratresorption aus dem Darm auf dem Lymphwege stattfindet; er hält den Zuckergehalt der Lymphe für eine sekundäre Folge der Blutzuckersteigerung.

*Borodulin* wiederholte die *Gigonschen* Versuche an mit Morphinum und Äther narkotisierten Hunden. 20 g einer 30-%igen Glykoselösung wurden direkt in den Darm eingespritzt. *Borodulin* fand da, dass der aus dem Dünndarm durch die V. portae kommende Zucker teilweise in der Leber zurückblieb und zum Teil unmittelbar in den grossen Kreislauf gelangte, wo derselbe eine Hyperglykämie hervorrief. Der in der V. hepatica auftretende Zucker soll nach *Borodulin* nicht aus dem Leberglykogen stammen. Ebensovienig soll die Narkose den Zuckerumsatz der Leber beeinflussen.

*Satake* und *Fujii* sowie *Conti* haben ähnliche Versuche an-

gestellt. Sie konstatierten, dass die Blutzuckerdifferenz in der V. portae und V. hepatica bei intestinaler Zufuhr von Glykose dieselbe ist wie bei intravenöser.

*Eisner* fand bei Versuchen an Kaninchen mit Glykosezufuhr durch eine Magensonde schon nach 3 Min. eine Hyperglykämie. In der V. portae war die Blutzuckerzunahme rascher und stärker als im peripheren Blut. Bei ähnlichen Versuchen mit Lävulose liess sich eine deutliche Blutzuckererhöhung im Venenblut bereits nach 1—1½ Min. beobachten. Nach 3 Min. konnte Lävulose im Blut der V. portae und nach 4 Min. im peripheren Venenblut chemisch nachgewiesen werden. Dabei ergab sich ein höherer Blutzuckergehalt, als es der Lävulosemenge entsprechen würde. *Eisner* nahm an, dass sowohl eine Reizungs- als auch eine Resorptionshyperglykämie vorlag. Das Überwiegen der einen oder anderen Art soll u. a. vom Füllungsgrad des Magens und Darmkanals sowie vom funktionellen Zustand des vegetativen Nervensystems abhängen.

*Kronberger* und *Radt* führten ähnliche Lävuloseversuche an Kaninchen aus, welche 24 Stunden lang gehungert hatten. Schon nach 2—5 Min. liess sich Lävulose im Venenblut nachweisen, gewöhnlich in geringer Menge. Die Autoren konnten keinen bestimmten Parallelismus zwischen Blutzucker- und Lävulosekurve finden. Bei einem Versuch konstatierten sie Blutzuckersteigerung ohne Lävulose, bei einem anderen beruhte die Hyperglykämie fast ausschliesslich auf der Lävulose.

*Loeweneck* und *Wachsmuth* haben Ergotamin-Atropinversuche an Hunden mit Eckscher Fistel und nach peroraler Zufuhr von Lävulose vorgenommen. Sie stellten dabei fest, dass sich die postalimentäre Hyperglykämie mittels Ergotamin im wesentlichen unterdrücken liess, im Einklang mit den Postulaten der Reizungstheorie. Eine geringere Blutzuckersteigerung blieb jedoch bestehen, und diese entsprach der im Blute nachweisbaren Lävulosemenge. *Loeweneck* und *Wachsmuth* bemerken indessen, dass sie die angewandte kolorimetrische Methode nach *van Creveld* in gewissen Fällen als nicht spezifisch befunden haben, da sie auch ohne Lävulosezufuhr einen positiven Ausschlag geben konnte.

*Depisch* und *Hasenöhl* stehen wie *Barrenscheen* und *Eisler*

u. a. auf dem Standpunkt, es müsse zwei Typen von normalen postalimentären Hyperglykämiekurven geben, nämlich den *Resorptionstypus*, der von niedriger Hyperglykämie gekennzeichnet wird, ohne nachfolgende Hypoglykämie, und der wahrscheinlich ein Anzeichen gesteigerter Zuckerspeicherung in der Leber ist, sowie den *Reizungs- oder Assimilationstypus* mit starker und plötzlich einsetzender, von einer mehr oder weniger deutlichen hypoglykämischen Phase gefolgt Hyperglykämie. Dieser Typus beruhe wahrscheinlich auf erhöhter Mobilisierung von Leberglykogen mit dann folgender vermehrter Zuckerresorption in den Geweben als Reaktion.

*Aschenheim* weist wie *Horiuchi* auf die Bedeutung des Funktionszustands des Darmepithels für die postalimentäre Hyperglykämie hin. *Mertz* und *Rominger* versuchten experimentell Klarheit über den Zusammenhang zwischen alimentärer Hyperglykämie und Darmresorption durch Glykosebelastungsproben mit und ohne gleichzeitige Anwesenheit von Gerbsäure zu gewinnen. Sie fanden dabei so erhebliche Unterschiede, dass sie in Übereinstimmung mit *Schade* die Ansicht äusserten, der kolloidale Zustand der Darmschleimhaut und die Resorptionsverhältnisse im Darm wären für die Form der alimentären Hyperglykämiekurve entscheidend. Es ist jedoch nicht möglich, aus dem Blutzuckergehalt Rückschlüsse auf die Resorptionsverhältnisse im Darm zu ziehen, was schon *Bergmark* hervorgehoben hat (vgl. S. 79).

*Pollak* nimmt das Vorhandensein eines unbekannten Mechanismus an, durch dessen Wirkung der aus dem Darm auf dem Wege über die V. portae kommende Zucker quantitativ in der Leber Aufnahme finden kann, ohne dass der Zuckergehalt des peripheren Blutes zu steigen braucht. Normalerweise bildet dieser Mechanismus eine Schranke zwischen dem Kohlehydratstrom aus dem Darm und den peripheren Geweben, er wird aber nach *Pollak* durch Funktion des vegetativen Nervensystems ausgeschaltet.



## Der Blutzuckergehalt als Gleichgewichtszustand

Die Schwankungen des Blutzuckergehalts kommen durch Interferenz zahlreicher Faktoren zustande. Ein Blutzuckerwert ist daher an und für sich eine in hohem Grade komplexe Grösse. Neben der dem Blute zugeführten Glykosemenge ist für den Blutzuckerspiegel noch das Quantum von Glykose bestimmend, welches in der Zeiteinheit aus dem Blute verschwindet. Ein konstanter Blutzuckergehalt setzt ein Gleichgewicht zwischen zugeführter und dem Blute entzogener Zuckermenge voraus.

Die Glykose, welche aus dem Blute verschwindet, kann in Geweben und Organen verbrannt, als Glykogen in der Leber, den Muskeln und anderen Geweben gespeichert, oder evtl. in Fett umgewandelt werden, das als Depotfett gesammelt wird. Sie kann auch, in Organfett verwandelt, am Fett- und Lipoidumsatz der lebenden Zellen teilnehmen. Übersteigt der Zuckergehalt im Blut einen bestimmten Schwellenwert, so wird ausserdem eine gewisse Menge Glykose im Urin ausgeschieden.

Ein neurohormonaler Regulationsmechanismus, im VI. Abschnitt eingehender behandelt, hält den Blutzuckerspiegel unter normalen Verhältnissen im Bereich enger Grenzen; die Werte schwanken im Hunger zwischen 0,08 und 0,12 %, bei Nahrungszufuhr mit Blutzuckersteigerungen normalerweise bis 0,18 % (s. Abb. 13).

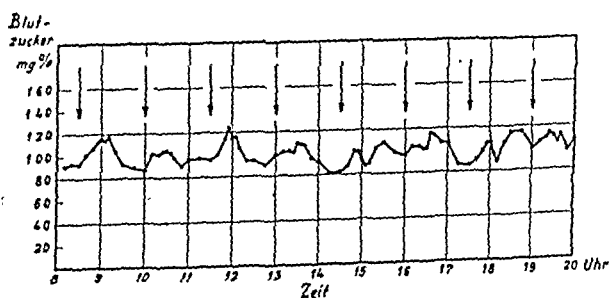


Abb. 13. Normale Blutzuckervariationen während des Tages bei Nahrungszufuhr. Die Pfeile geben einheitliche Mahlzeiten an (7,4 g Eiweiss, 17,3 g Fett und 21,6 g Kohlehydrate pro Mahlzeit).

Der normale Blutzucker ist eine reduzierende Hexose, welche man für eine Form der Glykose hält (vgl. S. 98). Der Blutzuckergehalt wird daher gewöhnlich durch das Reduktionsvermögen der Hexose bestimmt. Bei Störungen im Kohlehydratumsatz, z. B. bei gewissen Diabetesformen, treten auch andere reduzierende Stoffwechselprodukte, die nicht den Charakter der Hexosen haben, auf, was die Bestimmung der wirklichen Hexose erschwert. Eine Voraussetzung für die einwandfreie Feststellung des Zuckergehalts im Blute ist daher in vielen Fällen eine genauere Analyse der Kohlehydrate des letzteren. Da eine derartige Analyse beim klinischen Blutzuckerstudium zur Zeit in grösserem Umfang nicht stattfindet, wird noch immer die Bezeichnung »Blutzucker« als ein *Sammelbegriff für die reduzierenden Kohlehydrate des Blutes, welche nicht mit dem Bluteiweiss ausgefällt werden, verwendet*. Unter normalen Verhältnissen entspricht der Glykosegehalt des Blutes diesem Begriff ganz gut. Beim Diabetes dagegen können andere reduzierende niedermolekulare Kohlehydrate vorhanden sein, welche Glykose ganz oder teilweise ersetzen und im Energiehaushalt des Organismus eine Rolle spielen. Hier- von wird im VIII. Abschnitt S. 286 noch die Rede sein.

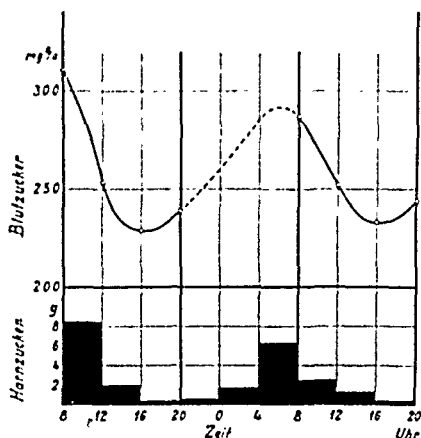


Abb. 14. Blutzuckervariationen während der 24 Stundenperiode im Hunger bei einem Fall von Diabetes (nach Hallehol).

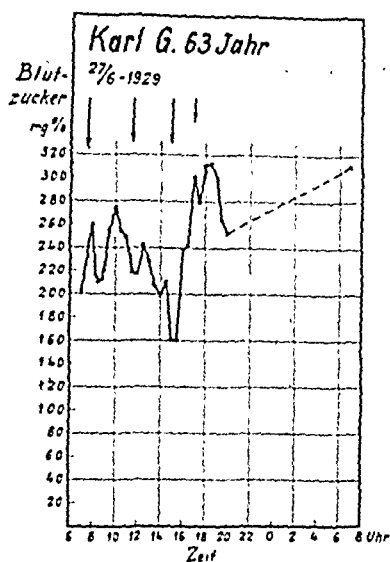


Abb. 15. Spontane Blutzuckersenkung bei Diabetes. Trotz Nahrungszufuhr sinkt der Blutzuckerspiegel um 15 Uhr auf unter dem Morgennüchternwert liegende Werte. Die Pfeile geben Mahlzeiten an. Kein Insulin.

beobachtet, welche ihm ein Rätsel waren. Der Blutzuckergehalt kann dabei trotz Nahrungszufuhr auf Werte sinken, welche den Nüchternwert unterschreiten (Abb. 15).

In anderen Fällen treten spontane Senkungen weniger hervor (Abb. 16).

Die Neigung zu spontanen Blutzuckersenkungen schwankt von Tag zu Tag. Dieselben machen sich beim Diabetes am deutlichsten bemerkbar, wenn die Kohlehydratzufuhr der Toleranzgrenze entspricht. Bei stärkerer Kohlehydratbelastung können die spontanen Senkungen verschwinden, man findet hierbei aber an den betreffenden Zeitpunkten eine veränderte Reaktion auf zugeführte Nahrung.

Dies zeigt sich da entweder in einer geringeren und rasch vorübergehenden postalimentären Blutzuckersteigerung oder als eine verspätete glykämische Reaktion bei Nahrungszufuhr (vgl. Abb. 17).

Bei Störungen der neurohormonalen Regulation im Zusammenhang mit Veränderungen im Kohlehydratumsatz machen sich mehr oder weniger starke Fluktuationen des Blutzuckergehalts bemerkbar.

Bei Fällen von schwerem Diabetes fand *Hatlehol* (1924) an Hungertagen einen regelmässigen 24stundenperiodischen Wechsel des Blutzuckergehalts, mit in den späten Nachtstunden steigenden, während des Tages aber sinkenden Werten (Abb. 14).

*Hatlehol* bemerkte einen gewissen Zusammenhang zwischen dem Schlaf und diesen Veränderungen des Blutzuckerspiegels.

Bei Nahrungszufuhr hatte schon *Petrén* (1923) bei gewissen Diabetikern spontane Blutzuckersenkungen

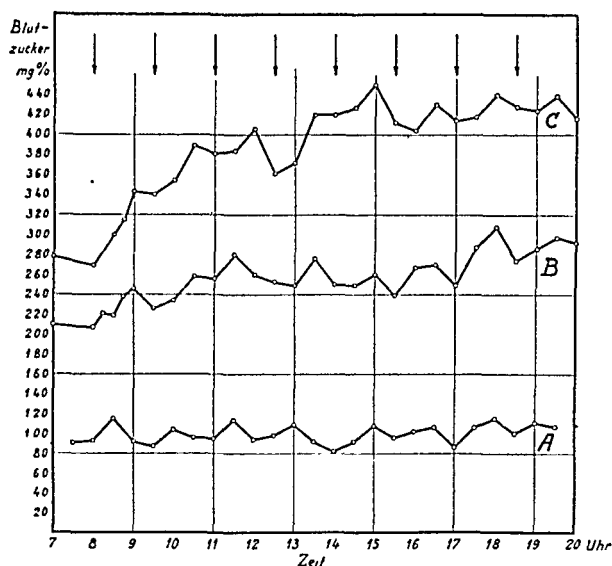


Abb. 16. Blutzuckercurven bei Nahrungszufuhr. Die Pfeile bezeichnen einheitliche Mahlzeiten (7,4 g Eiweiss, 17,3 g Fett und 21,6 g Kohlehydrate pro Mahlzeit). a normale Blutzuckerregulation. b mittelschwerer Diabetes. c schwerer Diabetes.

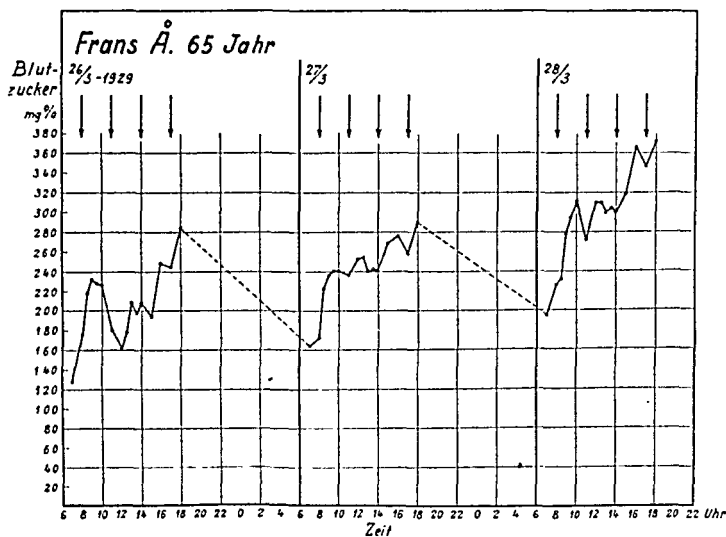


Abb. 17. Tagesvariationen des Blutzuckergehalts bei Diabetes. Die Pfeile geben einheitliche Mahlzeiten (24,4 g Eiweiss, 37,2 g Fett und 32,1 g Kohlehydrate pro Mahlzeit) an. Am ersten Tage (26.3.) macht sich eine spontane Blutzuckersenkung mit einem Minimum um 12 Uhr bemerkbar. Am nächsten Tage herabgesetzte glykämische Reaktion während der Zeit 10–14.

## Der Einfluss des Leberhythmus auf den Blutzuckergehalt

Für den die Leber verlassenden Blutzuckerstrom ist das Verhältnis zwischen Bildung und Abbau des Leberglykogens bestimmend. Während der assimilatorischen Phase der Lebertätigkeit ist die Glykogenbildung stärker als der Zerfall. Da hat der Blutzuckerspiegel die Tendenz zu sinken. Während der Dissimilationsphase dagegen erfolgt der Abbau lebhafter als die Neubildung. Der Blutzuckergehalt hat da eine Neigung zum Steigen. Diese dem Blutzuckergrundstrom entsprechenden Blutzuckerfluktuationen treten auch ohne Nahrungszufuhr auf und decken sich mit den von *Hatlehol* bei schwerem Diabetes gefundenen periodischen Blutzuckersteigerungen im Hunger.

Bei intensiverer Glykogensynthese nimmt die Fähigkeit der Leber, Kohlehydrate zurückzuhalten, zu; die *Kohlehydrataffinität* der Leber steigt. Während des Dissimilationsstadiums sinkt dagegen die Kohlehydrataffinität. Infolge der grossen Glykogenkapazität der Leber ist daher die rhythmische Leberfunktion ein wichtiger Faktor, der manchmal für den Wechsel des Blutzuckergehalts entscheidend werden kann. Die spontanen Blutzuckervariationen, welche sich beim Diabetes bisweilen sowohl im Hunger als auch bei Nahrungszufuhr beobachten lassen, sind wahrscheinlich ein Ausdruck von Schwankungen in der Kohlehydrataffinität der Leber.

Der Verlauf der Blutzuckerkurve nach Nahrungszufuhr ist teilweise von der Lage des Zeitpunkts, auf welchen die Mahlzeit fällt, im Verhältnis zur assimilatorischen oder dissimilatorischen Tätigkeitsphase der Leber abhängig. In dem einen Falle tritt eine rascher vorübergehende Blutzuckersteigerung ein, evtl. von einer hypoglykämischen Phase von dem Typus gefolgt, welcher als eine Reizungshyperglykämie charakterisiert worden ist. Im anderen bekommt die Hyperglykämie einen protrahierteren Verlauf vom Typus der Resorptionshyperglykämie.

Das Verhältnis der Blutzuckersteigerung zur rhythmischen Lebertätigkeit ist indessen ein komplizierteres Phänomen. Ne-

ben der grossen 24Stundenwelle der Lebertätigkeit laufen jene überlagernde kurzperiodische Schwankungen, was bei Betrachtung der in Abb. 3 wiedergegebenen Gallensekretionskurve klar ersichtlich wird. Da die Gallenabsonderung in gewissem Masse mit der Glykogenbildung abwechselt, muss auch die Leberassimilation kurzperiodischen Schwankungen unterworfen sein. Ausschlaggebend für den Verlauf der alimentären Blutzuckercurve wird da das zeitliche Verhältnis der Mahlzeiten zu diesen kurzperiodischen Variationen bei der Lebertätigkeit. Hierdurch erhält die alimentäre Blutzuckersteigerung in hohem Grade den Charakter einer *Interferenzkurve*. Eine geringfügige zeitliche Verschiebung der interferierenden Vorgänge — des Nahrungsreizes und der Lebertätigkeit — kann die Gestalt der Kurve in ganz verschiedener Weise verändern (vgl. Abb. 18).

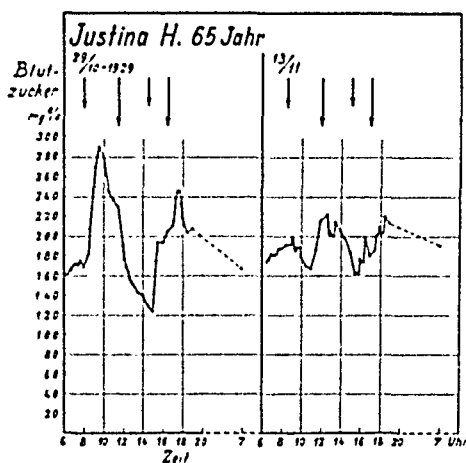


Abb. 18. Wechselnder Verlauf der Blutzuckerkurve bei Diabetes an verschiedenen Tagen bei genau gleicher Kost und Lebensweise infolge von Interferenz zwischen endogenem Rhythmus und Wirkung der Mahlzeiten. Die Pfeile bedeuten einheitliche Mahlzeiten. Man beachte die steigenden Nüchternblutzuckerwerte vor der ersten Mahlzeit.

Es ist aus diesem Grunde notwendig, durch mehrere Tagesversuche die kurzperiodischen Variationen der Lebertätigkeit soweit möglich auszugleichen, wenn man das Verhalten des Blutzuckers zur 24stundenrhythmischen Tätigkeit der Leber verfolgen will. Dies kann durch eine Serie von Tagesversuchen

TAB. 6.  
Veränderungen des Blutzuckergehalts an Probetagen sowie Ableitung der  $\Sigma$ -Differenzkurve.

Uhr	Blutzucker in mg %											
	7.0		8.0		9.30		11.0		12.30		14.0	
	Diff.		Diff.		Diff.		Diff.		Diff.		Diff.	
29.IV.29. 24 halbstündliche Mahlzeiten 8-19.30 .....	119	+40	159	+44	203	-13	190	+15	205	+17	188	+10
15.VIII.29. 8 Mahlzeiten (8.00, 9.30, 11.00, 12.30, 14.00, 15.30, 17.00, 18.30) .....	134	+35	169	+53	222	+11	233	-23	210	-16	194	+11
14.VI.29. 4 Mahlzeiten (8.00, 9.30, 12.30, 15.30) .....	164	+1	165	+58	223	-1	222	-48	174	+20	194	-31
21.VI.29. 4 Mahlzeiten (8.00, 12.30, 15.30, 18.30) .....	154	+47	201	+26	227	-61	166	-46	120	+60	180	-23
4.VI.29. 4 Mahlzeiten (8.00, 11.00, 14.00, 17.30) .....	149	+24	173	+56	229	-43	186	+7	193	-15	178	+2
$\Sigma$ -Diff.		+147		+237		-107		-95		+32		-31
								+81		+20		-41

Veränderung des Nüchternblutzuckergehalts in einer Stunde von 7 bis 8 Uhr..... +147  
 $\Sigma$ -Differenzkurve bei Nahrungszufuhr .....

.....zurechnende Zuckeraus-

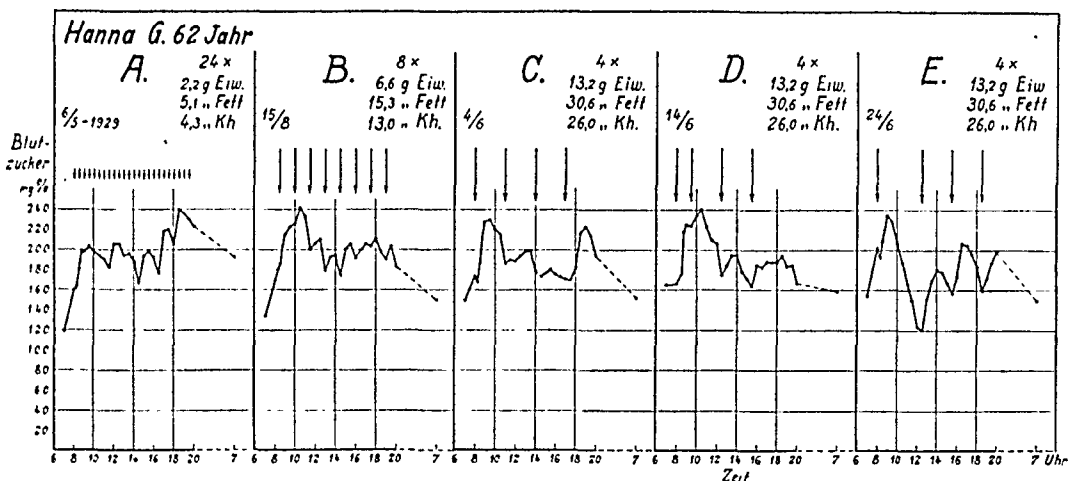


Abb. 19. Blutzuckercurven von fünf Tagesversuchen zur Berechnung der  $\Sigma$ -Differenzkurve und der endogenen Variationstendenz des Blutzuckergehalts.

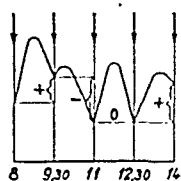


Abb. 20. Schema zur Berechnung von Plus- und Minusdifferenzen.

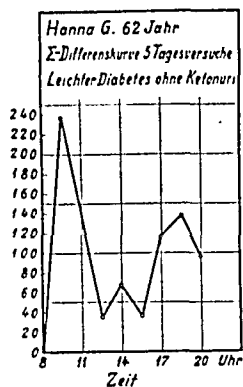


Abb. 21.  $\Sigma$ -Differenzkurve von fünf Tagesversuchen in Abb. 19.

geschehen, bei welchen die gleiche Nahrungsmenge auf eine wechselnde Anzahl von im Verhältnis zueinander verschobenen Mahlzeiten verteilt wird (Tab. 6 u. Abb. 19).

Dabei erhält man eine Anzahl Blutzuckercurven mit wechselndem Verlauf. Wenn diese Blutzuckercurven mit Rücksicht auf die Blutzuckerdifferenz an bestimmten Zeitintervallen analysiert (Abb. 20) und die Differenzen von korrespondierenden Zeitabschnitten summiert werden, erhält man eine  $\Sigma$ -Differenzkurve, in der zufällige Störungen ausgelöscht sind, wo aber die konstant vorkommende, sämtlichen Kurven gemeinsame Variationstendenz deutlicher zum Vorschein kommt.

Wenn Veränderungen des Blutzuckergehalts nur von alimentären Impulsen bestimmt werden, wird die  $\Sigma$ -Differenzkurve bei Verschiebung der Mahlzeiten gegeneinander eine gerade Linie. Sind dagegen endogene Faktoren periodischen Charakters



für den Verlauf der Blutzuckerkurve mitbestimmend, so spiegelt sich dies in der  $\Sigma$ -Differenzkurve wider. Eine derartige Untersuchungsserie wird in verkürzter Form in der Tabelle 6 Seite 70 und in vollständigerer Diagrammform in Abb. 19 wiedergegeben. Die Mahlzeiten werden aus dem Diagramm und Tabelle ersichtlich. Bei diesem Fall bestand keine Ketonurie.

Die  $\Sigma$ -Differenzkurve (Abb. 21) zeigt bei diesem Fall, dass der Blutzuckergehalt während der Zeit 13—15 Uhr weniger von resorbierter Nahrung beeinflusst wird als während der übrigen Zeiten am Tage. Auch die Zuckerausscheidung verschwindet in dieser Zeit. Dies deutet darauf hin, dass der Organismus da eine gesteigerte Neigung hat, Kohlehydrate zurückzuhalten.

Bei zwei Kontrollversuchen erhielt dieselbe Versuchsperson die gesamte Nahrungsmenge für die 24Stundenperiode in einer Mahlzeit, einmal um 8 Uhr, das andere Mal um 13 Uhr. Beide Male trat eine alimentäre Blutzuckersteigerung ein (vgl. Abb. 22). Die Blutzuckerkurve bekommt jedoch an den beiden Tagen einen verschiedenen Verlauf. Wenn die Mahlzeit um 8 Uhr gegeben wird, folgt die maximale Blutzuckersteigerung bereits binnen zwei Stunden, wonach der Blutzuckerspiegel ununterbrochen sinkt. Wird die Mahlzeit dagegen um 13 Uhr verabreicht, so tritt nach der initialen raschen Blutzuckerzunahme eine fortgesetzte langsame Steigerung ein, wobei der Blutzuckergehalt sein Maximum erst nach fünf Stunden, um 18 Uhr, erreicht. Dies stimmt auch mit dem Verlauf der  $\Sigma$ -Differenzkurve in Abb. 21 überein.

Das Verhalten der Zuckerausscheidung während der beiden Kontrolltage (Abb. 22) liefert einen weiteren Beleg für die Annahme, dass in diesem Fall die Reaktion des Organismus auf die zugeführte Nahrung am Morgen und in den Mittagstunden verschieden ist. Wird die Mahlzeit um 8 Uhr gegeben, so tritt eine rasch vorübergehende Zuckerausscheidung auf, welche 1,8 g in 2 Stunden entspricht. Fällt dagegen die Mahlzeit auf 13 Uhr, dann wird der Verlauf der Zuckerausscheidung protrahiert, und die ausgeschiedene Menge beträgt in diesem Falle nur 1,4 g während 5 Stunden, in völliger Übereinstimmung

mit dem Verlauf der Zuckerausscheidung in der  $\Sigma$ -Differenzkurve aus den fünf Tagesversuchen (Abb. 21).

Bei schwereren Formen von Diabetes mit hochgradiger Ketonurie bekommt die  $\Sigma$ -Differenzkurve bei Nahrungszufuhr einen gleichmässiger bis auf einen asymptotischen Grenzwert während des Tages steigenden Verlauf (Abb. 23).

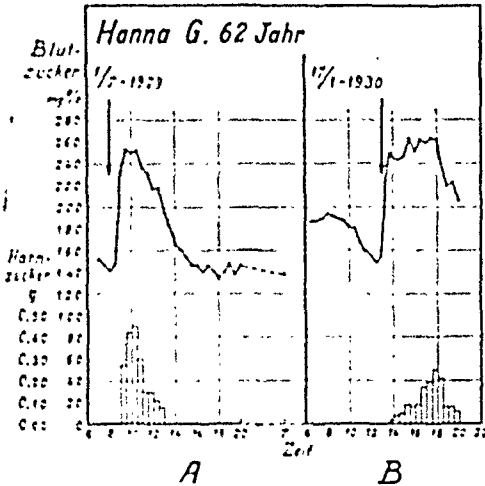


Abb. 22. Zwei Kontrollversuche für die  $\Sigma$ -Differenzkurve in Abb. 21. Die gesamte Nahrungsmenge wurde in einer Mahlzeit um 8 bzw. 13 Uhr verabreicht. Verlauf und Maximum der alimentären Blutzuckerkurve ähnlich der  $\Sigma$ -Differenzkurve (Abb. 21).

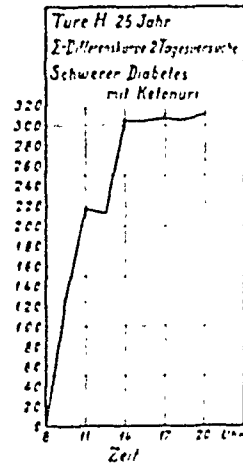


Abb. 23.  $\Sigma$ -Differenzkurve bei schwerem Diabetes mit starker Ketonurie.

*Diese Versuche lehren, dass die alimentäre Blutzuckersteigerung durch endogene Faktoren modifiziert wird, welche im Laufe des 24Stundenabschnitts periodisch wechseln. Der wechselnde Verlauf der alimentären Blutzuckerkurve sowie der verschiedene Charakter derselben (Resorptions- oder Assimilationstypus) ist von dem Zeitpunkt in bezug auf den endogenen Rhythmus und die periodische Lebertätigkeit abhängig, auf welchen die Mahlzeit fällt.*

Die Ketonkörperbildung findet in der Leber statt und setzt beim Sinken des Leberglykogengehalts ein (vgl. S. 151 ff.). Bei



der Leber, Kohlehydrate zurückzuhalten, ab. Die Zuckerdiurese geht deshalb in der Regel den Schwankungen des Blutzuckerspiegels parallel. Aus diesem Grunde variiert bei einem Diabetiker auch bei gleichbleibender Nahrungszufuhr die Zuckerausscheidung von Tag zu Tag, je nach den endogenen Umstellungen des Organismus mit den wechselnden Reaktionen auf zugeführte Nahrung. Ein diesbezügliches Beispiel gibt die Tabelle 7; bei diesem Versuch wurde die Zuckerausscheidung während längerer Zeit in bestimmten Abständen verfolgt.

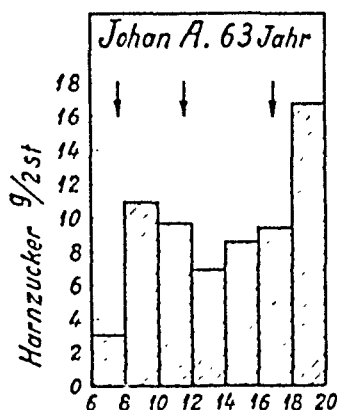


Abb. 24. Zuckerausscheidungsdiagramm bei Diabetes. Mittelwerte von 23 Tagen. Die Pfeile bezeichnen Mahlzeiten.

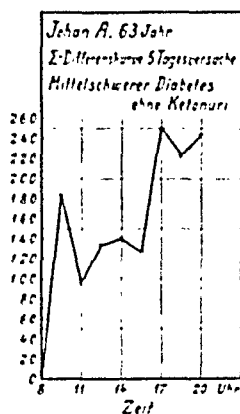


Abb. 25.  $\Sigma$ -Differenzkurve bei mittelschwerem Diabetes ohne Ketonurie, dem Zuckerausscheidungsdiagramm in Abb. 24 entsprechend. Zu beachten ist die Übereinstimmung zwischen Zuckerdiurese und Variationstendenz des Blutzuckergehalts während der fünf Versuchstage mit bestimmter Verteilung der Mahlzeiten.

Bildet man die Summen der in korrespondierenden Zeitabschnitten ausgeschiedenen Zuckermengen, so kommt der 24-stundenperiodische Charakter der Zuckerausscheidung zum Vorschein, sowie auch der verschiedene Einfluss von an verschiedenen Tageszeiten eingenommenen Mahlzeiten (Abb. 24).

Der Verlauf der  $\Sigma$ -Differenzkurve bei derselben Versuchsperson wird in Abb. 25 wiedergegeben.

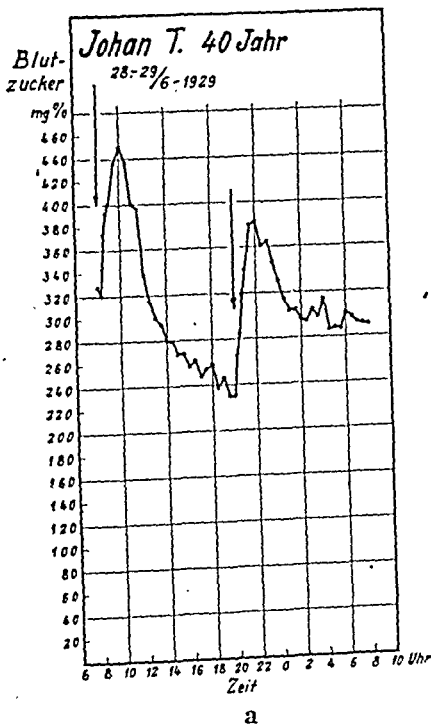
Bei Glykosurien vom renalen Typus besteht kein Parallelismus zwischen der Zuckerausscheidung und den Schwankungen



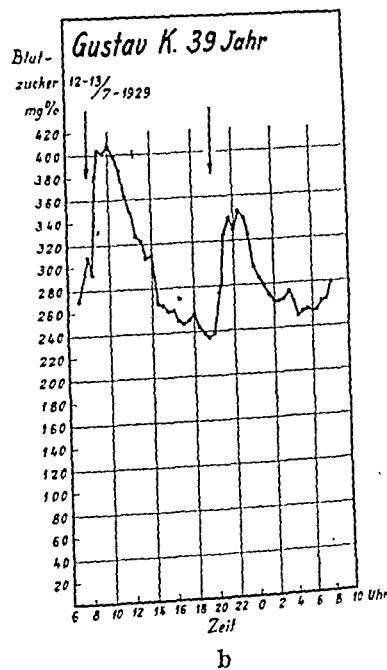


24Stundenperiode ausgeschiedene Zuckermenge kleiner, und die Differenz zwischen der Zuckerausscheidung am Tage und in der Nacht nimmt ab (vgl. Abb. 27 b).

Ihrem Wesen nach ist die Blutzuckerkurve eine Interferenzkurve. Dies ist der Grund dafür, dass sich auch die tägliche Zuckerausscheidung trotz einheitlicher Ernährung und sonst gleichen äusseren Bedingungen von Tag zu Tag ändert (vgl. Tab. 7). Dabei lässt sich bisweilen bei gewissen Diabetesfällen in der Zuckerausscheidung eine mehr oder minder deutliche 3—5tägige Periode auch bei gleichbleibender Nahrungszufuhr konstatieren.



a



b

Abb. 27. Verlauf der alimentären Blutzuckerkurve nach einer Mahlzeit am Morgen und am Abend. Die Pfeile geben die Mahlzeiten an (gleiche Zusammensetzung, 26,6 g Eiweiss, 61,2 g Fett und 52,0 g Kohlehydrate).

a Fall von mittelschwerem Diabetes ohne Ketonurie.  
Zuckerausscheidung:

8—20 Uhr 62,7 g

20—8 » 42,4 g. Differenz zwischen Tag und Nacht 20,3 g.

b Fall von schwerem Diabetes mit Ketonurie.  
Zuckerausscheidung:

8—20 Uhr 84,1 g

20—8 » 79,4 g. Differenz zwischen Tag und Nacht 4,7 g.

Alles das deutet darauf hin, dass die Reaktion des Organismus auf zugeführte Nahrung von endogenen Faktoren beherrscht wird. Es ist dabei nicht nur die periodische Glykogenbildung der Leber, welche bestimmend ist, sondern es gibt noch andere endogene Faktoren periodischen Charakters, welche gemeinsam einen *endogenen Rhythmus* entstehen lassen. Eine gegenseitige Phasenverschiebung der Wirkung dieser periodischen Faktoren verursacht eine scheinbare Unregelmässigkeit des endogenen Rhythmus, wodurch die Reaktion auf den alimentären Reizungsimpuls von Tag zu Tag wechselt, und die postalimentäre Blutzuckerkurve einen variablen, unregelmässigeren Verlauf nehmen kann. Dies macht sich beim Diabetes deutlich bemerkbar, wo die regulatorischen Kräfte beeinträchtigt sind. Die Beachtung dieses Sachverhalts ist bei der Beurteilung alimentärer Belastungsproben von allergrösster Bedeutung. Die zugeführte Nahrung kann an verschiedenen Zeiten wechselnde alimentäre Blutzuckersteigerungen auslösen, was auf verschiedenen Reaktionszuständen des Körpers beruht. Der Reaktionszustand wird seinerseits durch die Interferenz zwischen den periodisch variierenden endogenen Faktoren bestimmt, welche alle zusammen den endogenen Rhythmus verursachen, der den Stoffwechsel beherrscht und für die Wirkung zugeführter Nahrung und äusserer Reizungsimpulse ausschlaggebend ist (vgl. S. 273).

## Der periodische Verlauf der Resorption

Periodisch ablaufende Vorgänge machen sich bereits bei der Resorption von Nahrungsstoffen im Darmkanal bemerkbar. Im II. Abschnitt ist von der Periodizität der Fettresorption die Rede gewesen. Auch die Glykoseresorption im Dünndarm spielt sich nach Untersuchungen von *Hamar* (1940) periodisch ab. Das Maximum der Fettresorption scheint mit dem der Glykoseresorption zusammenzufallen.

Die periodisch wechselnde Resorptionsfähigkeit des Darmepithels kann möglicherweise mit der Tätigkeit der Nebennieren zusammenhängen; nach *Verzár* und *Laszt* hört die Fett-



resorption nach Nebennierenexstirpation auf. Auch die Resorption der Kohlehydrate scheint von den Nebennieren abhängig zu sein, aber darüber ist nichts mit Sicherheit bekannt.

Ferner ist auch die Hypophyse für die Resorption von sowohl Glykose wie Fett von Bedeutung. *Philipps* und *Robb* haben nachgewiesen, dass die Glykoseresorption nach Entfernung der Hypophyse ebenso sinkt wie nach Nebennierenexstirpation.

Die Wirkung der Hypophyse auf die Kohlehydrat- und Fettresorption wird wahrscheinlich von dem corticotropen Hypophysenvorderlappenhormon vermittelt, das seinerseits die Bildung des Nebennierenrindenhormons auslöst, dessen Effekt für die Resorption jener Nährstoffe nötig zu sein scheint.

Das Nebennierenrindenhormon spielt anscheinend nicht nur bei der eigentlichen Resorption eine wichtige Rolle, sondern ausserdem noch bei der Bildung von Muskel- und namentlich Leberglykogen. *Long*, *Katzin* und *Fry* (1940) haben gezeigt, dass sich auch die Hungerhypoglykämie bei hypophysenlosen Tieren durch Behandlung mit Nebennierenrindenhormon oder Corticosteron verhindern lässt. Unter dieser Behandlung nimmt das Leberglykogen sowie ferner das Muskelglykogen zu. Gleichzeitig steigt die Stickstoffausscheidung im Urin, was auf eine Kohlehydratbildung aus Eiweiss hindeutet. Das Eingreifen der Hypophyse in den Zuckerstoffwechsel wird weiterhin im VI. Abschnitt im Zusammenhang mit der Frage der neurohormonalen Blutzuckerregulation behandelt werden.

Der Kohlehydratstrom in das Blut erfolgt also einerseits durch die Resorption aus dem Darmkanal und andererseits durch den Zerfall des Leberglykogens in periodischem Wechsel. Der Blutzuckergehalt wird aber nicht nur durch die Glykosezufuhr bedingt, sondern auch durch die Menge des das Blut verlassenden Zuckers. Dies geschieht auf vielen verschiedenen Wegen, welche im VI. Abschnitt näher besprochen werden sollen.

Ein starker Glykoseverbrauch findet in der Muskulatur gelegentlich der Muskelarbeit statt. Die Frage des Einflusses der Muskelarbeit auf den Blutzuckerspiegel besitzt daher spezielles Interesse.

## Blutzuckergehalt und Muskularbeit

Weiland hatte schon 1908 nachgewiesen, dass Muskularbeit den Blutzuckergehalt senken kann. Bürger fand 1914, dass körperliche Arbeit bei gesunden Personen auch eine initiale Blutzuckersteigerung auslösen konnte, eine regulative Arbeitshyperglykämie, auf welche eine vorübergehende Blutzuckersenkung folgen konnte. In gewissen Fällen trat nach dieser Senkung eine erneute Blutzuckerzunahme auch ohne Nahrungszufuhr auf.

Lichtwitz beobachtete bei Diabetikern ein starkes Steigen des Blutzuckerspiegels nach körperlicher Anstrengung. Dies wurde von vielen Autoren bestätigt (Moraczewski, Brösamlen und Sterkel, Strandell u. a.). In gewissen Fällen bleibt indessen die Arbeitshyperglykämie beim Diabetes aus, und statt dieser tritt eine Abnahme des Blutzuckergehalts ein (Lichtwitz, Zachariae, Lillie u. a.).

Bei allen älteren Untersuchungen ist die periodische Glykogenbildung in der Leber unberücksichtigt geblieben. Um zur Klarheit über die Frage des Einflusses der Muskularbeit auf den Blutzuckergehalt zu gelangen, ist es unumgänglich, dass auch die Schwankungen des Blutzuckerspiegels im Zusammenhang mit der rhythmischen Leberfunktion beachtet werden.

Möllerström und Ullmark haben die Veränderungen des Blutzuckergehalts bei Gesunden teils bei Muskularbeit und teils in der Ruhe an verschiedenen Zeiten der 24Stundenperiode untersucht, und zwar sowohl im Hunger als auch bei kontinuierlicher peroraler Glykosezufuhr (Abb. 28).

Die Ergebnisse dieser Untersuchungen sind in Kürze folgende:

1. Bei normaler Blutzuckerregulation ohne Nahrungszufuhr hat auch eine intensive Muskularbeit bloss unerhebliche Blutzuckervariationen zur Folge. Nach der Arbeitsperiode ist der Blutzuckerspiegel in der Hauptsache unverändert.

2. Bei kontinuierlicher Glykosezufuhr per os während der Versuchsdauer ruft Muskularbeit bei Nichtdiabetikern eine vorübergehende Senkung des Blutzuckers hervor, welche an

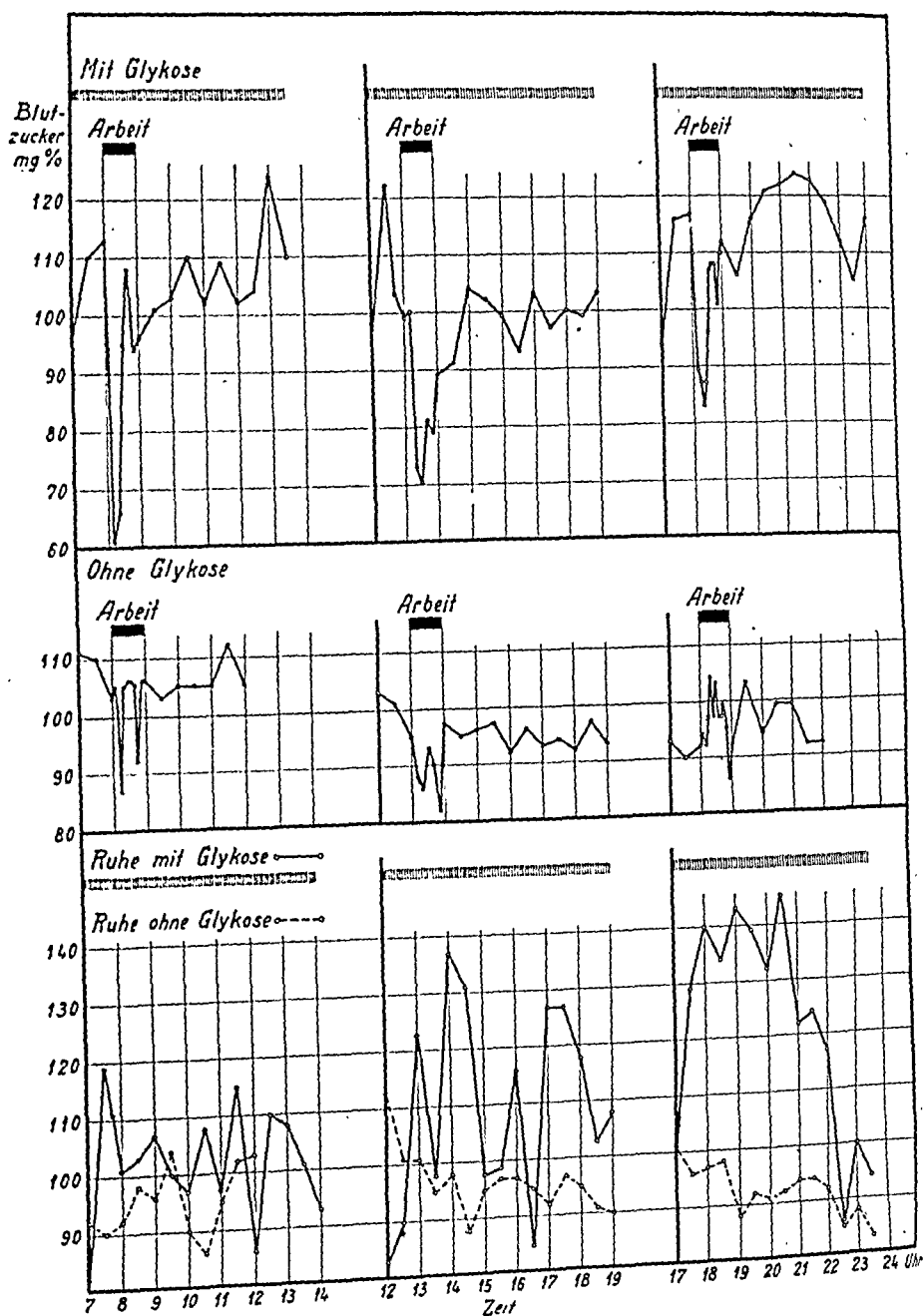


Abb. 28. Veränderungen des Blutzuckerspiegels bei Muskelarbeit im Hunger und bei kontinuierlicher Nahrungszufuhr. Die Arbeitsleistung entsprach 56 000 kgm (Fahrradergograph) während einer Stunde. Bei den Hungerversuchen letzte Mahlzeit fünf Stunden vor Beginn des Versuchs (60 g Brot, 400 g Milch, 50 g Butter, 20 g Käse). Bei den Versuchen mit kontinuierlicher Nahrungszufuhr wurden während der ganzen Versuchsdauer alle fünf Minuten 10 ccm Glykoselösung gegeben (nach Möllerström und Ullmark).

verschiedenen Zeiten der 24Stundenperiode wechselt. Eine mässige Arbeitsleistung (27 000 kg/st) bewirkt nur eine geringe Blutzuckersenkung, wird aber die Arbeit auf das Doppelte gesteigert, so wird die Blutzuckersenkung erheblich stärker.

3. Bei nach Schluss der Arbeitsperiode fortgesetzter Glykosezufuhr tritt eine Blutzuckersteigerung auf, die an verschiedenen Tageszeiten wechselt.

Bemerkenswert ist, dass Muskelarbeit im Hunger normalerweise keine nennenswerten Blutzuckerveränderungen veranlasst. Wird dagegen Glykose kontinuierlich zugeführt, so erfolgt an gewissen Zeiten der 24Stundenperiode eine auffällige temporäre Blutzuckersenkung, mit niedrigeren Werten als bei den entsprechenden Hungerversuchen. Die Ursache ist noch nicht geklärt, aber dieses Phänomen kann ein manchmaliges paradoxes Auftreten eines Insulinschocks bei Diabetikern selbst während einer Mahlzeit nach vorangehender intensiver Muskelanstrengung erklären. Diese Fragen müssen weiter mit spezifischen Methoden in Angriff genommen werden (vgl. S. 286).

## Insulinwirkung und endogener Rhythmus

Die blutzuckersenkende Wirkung des Insulins ist von u. a. *Best, Dale, Hoet* und *Marks* mittels direkter Durchströmungsversuche studiert worden. Dabei wurden Sauerstoff- und Glykoseverbrauch der Organe sowie der Glykogengehalt der Leber und Muskulatur vor und nach der Insulinwirkung bestimmt. Man fand bei diesen Versuchen, dass der Oxydationsprozess gesteigert wird, wodurch die Glykoseverbrennung zunimmt. Ausserdem findet auch eine Speicherung von Muskelglykogen ohne entsprechende Vermehrung des Leberglykogens statt.

Durch die Wirkung des Insulins scheinen mithin Kohlehydrate aus der Leber zur Muskulatur transportiert zu werden.

Aus Untersuchungen von *London* und *Kotschnew* (vgl. S. 56) geht hervor, dass die Leber im Hunger lediglich Glykose an das Blut abgibt. Bei kohlehydratreicher Kost dagegen lie-

fert der Darm einen Glykoseüberschuss an das Blut ab. Dieser aus dem Darm stammende Zuschuss wird bei der Insulinwirkung kleiner. Möglicherweise treten die im Darm resorbierten Kohlehydrate unter dem Einfluss des Insulins in irgendeiner Form, evtl. als Glykogen, auf, welche sich den üblichen Blutzuckerbestimmungen entzieht.

*London* und *Kotschnews* Untersuchungen machen ferner ersichtlich, dass die arterio-venöse Blutzuckerdiffereenz bei Kohlehydratzufuhr und gleichzeitiger Insulinwirkung in den Nieren steigt, aber in der Leber und auch, doch in geringerem Grade, in der Muskulatur abnimmt. Da also der Zuckerverbrauch der Nieren bei der Insulinwirkung grösser wird, der Zuckerverbrauch in der Leber aber abnimmt und ausserdem die Muskulatur nach wie vor erhebliche Glykosemengen beansprucht, muss die Leber unter dem Einfluss von Insulin eine gewisse Menge Kohlehydrate abgeben und dabei von ihrem Glykogenvorrat zehren. Es kommt hierdurch unter der Einwirkung des Insulins zu einer Kohlehydratverschiebung aus der Leber zur Muskulatur, im Einklang mit den Beobachtungen von *Best*, *Dale*, *Hoet* und *Marks*. Infolge des verringerten Kohlehydratstroms in das Blut und des gesteigerten Glykoseverbrauchs, speziell in den Nieren, sinkt der Blutzuckerspiegel. Ist die Leber arm an Glykogen, wie es beim Übergang von der Dissimilationsphase zum assimilatorischen Funktionsstadium derselben der Fall ist, so kann Blutzucker bei der Insulinwirkung nicht in hinreichender Menge mobilisiert werden, weshalb der Blutzuckergehalt auf hypoglykämische Werte sinken kann, wobei ein Insulinschock mit Krämpfen, Schweissausbrüchen und evtl. Bewusstlosigkeit droht. Diabetesformen mit stark reduziertem Leberglykogengehalt sind daher sehr insulinempfindlich und reagieren leicht mit hypoglykämischen Symptomkomplexen.

Es ist nicht der Glykogengehalt der Leber allein, welcher für die Insulinempfindlichkeit entscheidend ist, sondern auch das Bestreben des Organs, Kohlehydrate zurückzuhalten. Die Kohlehydrataffinität der Leber spielt in dieser Beziehung eine Rolle. Diese ist am grössten, wenn die Leberassimilation am stärksten ist.

Holmgren und Möllerström haben die Insulinempfindlichkeit bei Kaninchen unter Berücksichtigung der rhythmischen Leberfunktion studiert. Durch Serien von Tagesversuchen mit und ohne Nahrungszufuhr sowie halbstündlichen Blutzuckerbestimmungen wurden zunächst die normalen Blutzuckerkurven der Versuchstiere ermittelt. Aus diesen Kurven wurden dann  $\Sigma$ -Differenzkurven berechnet, welche die endogene Va-

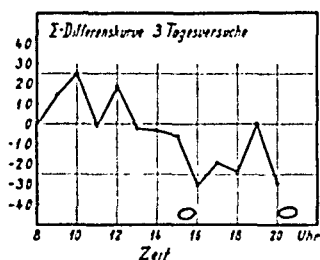


Abb. 29.  $\Sigma$ -Differenzkurve vom Kaninchen, durch drei Tagesversuche erhalten. An zwei Versuchstagen durfte das Versuchstier Heu, Hafer und Kohlrüben ohne Einschränkung fressen, am dritten Tage erhielt es kein Futter. Halbstündliche Blutzuckerbestimmungen nach Hagedorn während des Tages. Die Ovale bezeichnen Zeitpunkte, an welchen der Blutzuckerspiegel eine starke Tendenz zum Sinken hat.

riationstendenz des Blutzuckergehalts ersichtlich machen. In anderen Tagesversuchen an denselben Tieren wurde dann die Veränderung des Blutzuckergehalts nach an verschiedenen Tageszeiten gegebenen Insulindosen untersucht. In Abb. 30 ist ein vollständiger Versuch mit Insulinkurven für alle zwei Stunden während der 24Stundenperiode dargestellt.

In der  $\Sigma$ -Differenzkurve (Abb. 29) treten bei diesem Versuchstier zwei Zeitpunkte mit Neigung zu stärkerem Blutzuckerfall hervor, einmal um etwa 16 Uhr, sodann um ca. 20 Uhr. Im Einklang hiermit bekamen auch die Versuchstiere nach um 12 bzw. 14 Uhr gegebenen Insulindosen Insulinkrämpfe um 14.45 und 15.30 Uhr, sowie um 22 Uhr nach einer Insulindosis um 20 Uhr. Während sonstiger Zeiten des 24Stundenabschnitts traten keine hypoglykämischen Krämpfe auf, selbst wenn die Insulindosis gesteigert wurde.

An anderen Tageszeiten ist dagegen die Insulinempfindlichkeit gleichmässiger, und die Blutzuckersenkung nimmt oft

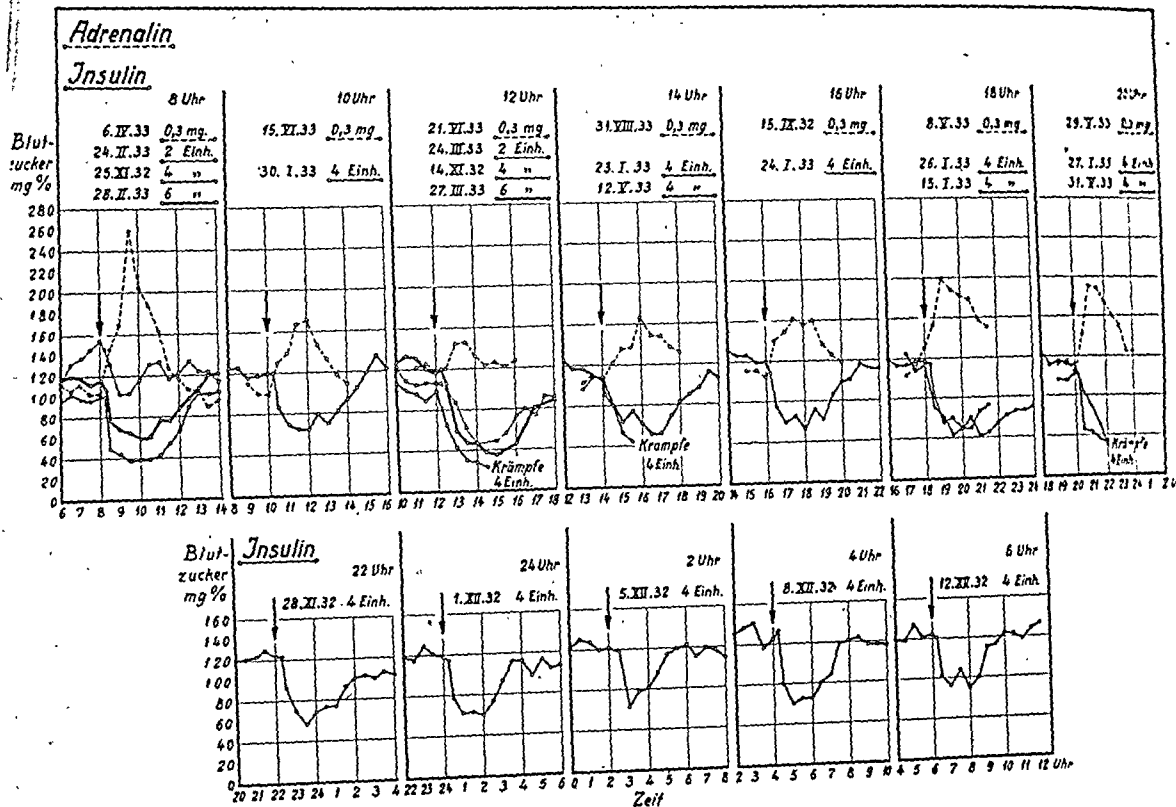


Abb. 30. Veränderungen des Blutzuckerspiegels nach Insulin und Adrenalin bei demselben Versuchstier wie in Abb. 29. Bei den einzelnen Stundenversuchen wurde zweistündlich während der 24 Stundenperiode Insulin in wechselnden Mengen (4, 6, 8 I.E.) gegeben und dann die Veränderungen des Blutzuckers 8 Stunden lang verfolgt. Während der Versuche erhielt das Tier kein Futter. Man beachte, dass hypoglykämische Krämpfe an den in Abb. 29 mit Ovalen bezeichneten Zeitpunkten auftreten (nach Holmgren und Möllerström).

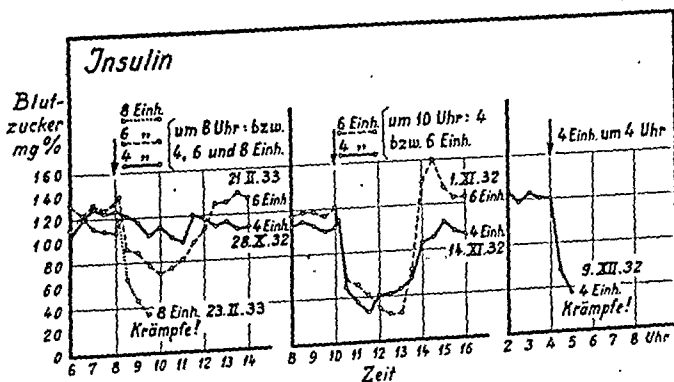


Abb. 31. Verschiedene Wirkung des Insulins an verschiedenen Tageszeiten beim Kaninchen. Um 4 Uhr wurden bei dem Versuchstier hypoglykämische Krämpfe von der Hälfte der um 8 Uhr gegebenen Insulinmenge ausgelöst. Zu beachten ist die maximale Blutzuckersenkung um 10 Uhr nach einer kleineren Insulindosis und die reaktive Hyperglykämie nach einer grösseren.

mit steigenden Insulinmengen zu. An gewissen Zeitpunkten kann jedoch eine kleinere Insulindosis eine maximale Blutzuckersenkung bewirken. Wird da die Insulindosis vergrößert, so tritt nur eine gesteigerte reaktive Hyperglykämie nach dem Blutzuckerfall ein (vgl. Abb. 31):

Die direkt blutzuckersenkende Wirkung des Insulins hält etwa vier Stunden an. Nachher steigt der Blutzuckerspiegel wieder.

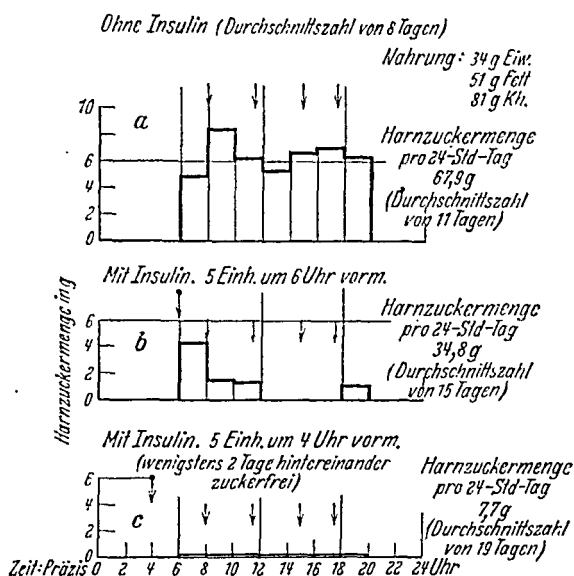


Abb. 32. Zuckerausscheidungsdiagramm bei Diabetes. Der Einfluss des Insulins auf die Zuckerdiurese wurde bei Verschiebung der Insulindosis um zwei Stunden mit Rücksicht auf den Ausscheidungsrythmus verdoppelt.

Auch der Einfluss des Insulins auf die Harnzuckerausscheidung hängt von dem endogenen Rhythmus ab. Abb. 32 macht ersichtlich, wie die Wirkung des Insulins in bezug auf die Zuckerausscheidung bei einem Fall von Diabetes nahezu verdoppelt wurde, wenn die Insulindosis zwei Stunden eher am Morgen gegeben wurde, unabhängig von Mahlzeiten. Dies erklärt die auch von *Ingolf* und *Tillgren* gemachte Beobachtung über die günstige Wirkung von in den frühen Morgenstunden verabreichten Insulindosen.



Die Wirkung des Insulins ist also an verschiedenen Tageszeiten nicht dieselbe. An gewissen Zeitpunkten ist der Organismus zu hypoglykämischen Zuständen disponiert. Dies hängt mit der rhythmischen Leberfunktion und dem wechselnden Glykogengehalt der Leber zusammen. *Forsgren* hat gezeigt, dass die Kaninchenleber bei Insulinkrämpfen regelmäßig glykogenarm ist.

Die Kenntnis der schwankenden Insulinempfindlichkeit des Organismus ist sowohl für eine rationelle Insulinbehandlung als auch für die Standardisierung von Insulinpräparaten von grösster Bedeutung.

## Adrenalinhyperglykämie und endogener Rhythmus

Die blutzuckersteigernde Wirkung des Adrenalins ist von dem Zeitpunkt der Injektion desselben im Verhältnis zur rhythmischen Leberfunktion abhängig. *Holmgren* und auch *Holmgren* und *Wohlfart* haben den Adrenalineffekt an verschiedenen Zeiten der 24Stundenperiode mittels ähnlicher Versuche untersucht. Die Blutzuckersteigerung nach ein und derselben Adrenalindosis wechselte dabei an verschiedenen Zeitpunkten ganz beträchtlich. Abb. 30 zeigt auch eine Serie von

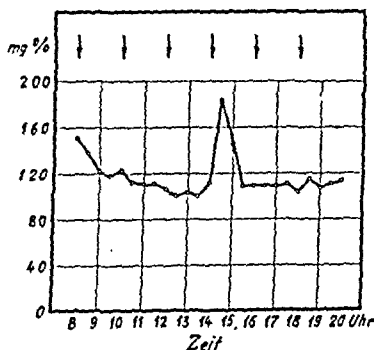


Abb. 33. Verlauf der Blutzuckerkurve beim Kaninchen bei wiederholten kleinen Adrenalindosen. Zweistündlich 0,1 mg Adrenalin, keine Fütterung. Die Pfeile bezeichnen die Adrenalininjektionen (nach *Holmgren*).

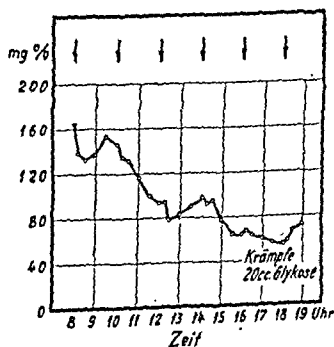


Abb. 34. Blutzuckerveränderungen beim Kaninchen nach wiederholten kleinen Insulingaben. Das hungernde Tier erhielt zweistündlich 1 i. E. Insulin subkutan. Die Pfeile geben die Insulininjektionen an (nach *Holmgren*).

Adrenalinbelastungsproben alle zwei Stunden während des Tages.

Einen anderen Versuchstypus veranschaulicht Abb. 33. Hier wurden während des Tages kleinere Insulin- bzw. Adrenalin-dosen in gleichen Zeitabständen gegeben. Es ergibt sich da, dass an einem bestimmten Zeitpunkt eine spezielle Empfindlichkeit gegen Adrenalin besteht, welches nun eine starke Hyperglykämie bewirkt. An demselben Zeitpunkt findet man eine Blutzuckersteigerung auch bei wiederholten kleinen Insulindosen (Abb. 34).

Auch in Normalkurven ohne Nahrungszufuhr tritt am entsprechenden Zeitpunkt eine Blutzuckersteigerung hervor (Abb. 35). Dies deutet auf spontane Schwankungen in der Regulation des Blutzuckerspiegels hin, welche von der Adrenalin- bzw. Insulinwirkung akzentuiert werden.

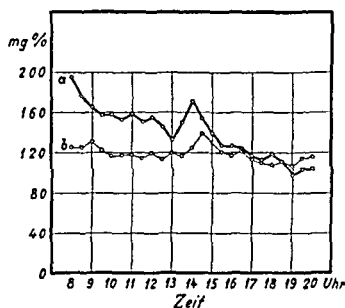


Abb. 35. Normale Blutzuckerkurven beim Kaninchen. Obere Kurve: Hunger vom Beginn des Versuchs an, untere: Hunger schon 12 Stunden vorher sowie während des Versuchs. Man beachte die Blutzuckersteigerung bei diesem Versuchstier um etwa 14 Uhr, an dem Zeitpunkt der Blutzuckersteigerung bei sowohl Insulin- wie Adrenalinversuchen (Abb. 33 u. 34 dasselbe Versuchstier). (Nach Holmgren.)

Der neurohormonale Regulationsapparat des Blutzuckers hat mithin an verschiedenen Zeiten der 24Stundenperiode nicht dieselbe Empfindlichkeit gegen Insulin bzw. Adrenalin. Es ist ein periodischer Wechsel, der möglicherweise mit dem sich ändernden Glykogengehalt der Leber direkt verknüpft ist, jedenfalls aber *unter normalen Verhältnissen mit der rhythmischen Lebertätigkeit und den Schwankungen der Kohlehydrataffinität dieses Organs harmonisch koordiniert ist*. Es

ist nicht möglich, zu entscheiden, ob die neurohormonale Periode oder die periodische Glykogenbildung bei der wechselnden Reaktion des Organismus auf alimentäre Reizimpulse, Insulin- und Adrenalinwirkung das Primäre ist. Der endogene Rhythmus ist von komplexer Natur und kommt durch Interferenz vieler periodisch wechselnder Faktoren zustande, gewissermassen wie sich eine Symphonie aus den Tongängen der verschiedenen Instrumente aufbaut.

## IV. ABSCHNITT

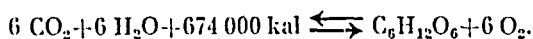
## Über die biochemischen Intermediärstufen des Stoffwechsels.

Die Voraussetzung für eine Vertiefung unserer Kenntnisse über Bildung und Zerfall des Glykogens ist ein Einblick in die physiologisch-chemischen Prozesse, welche den Kohlehydratumsatz im Organismus beherrschen. Wie die Stärkeerzeugung bei den Pflanzen erfolgt die Glykogenproduktion durch eine Synthese aus einfacheren Bestandteilen. Die Einzelheiten des Verlaufs sind nicht bekannt, aber die zur Synthese erforderliche Energie muss bei dem intermediären Umsetzungsprozess im Zusammenhang mit gekoppelten chemischen Reaktionsvorgängen frei werden. Die Glykogenbildung im Tierkörper und die Stärkeproduktion der Pflanzen sind in gewissem Sinne analoge Erscheinungen, nur ist der Vorgang bei der Bildung von Stärke in energetischer Beziehung insofern einfacher, als die Lichtenergie bei der Synthese ausgenützt wird.

## Die Stärkebildung bei Pflanzen

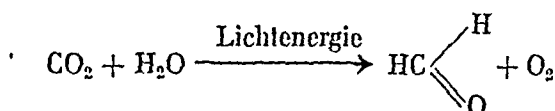
Bei der Kohlensäureassimilation entstehen in den grünen Pflanzenteilen Kohlehydrate. Durch Vermittlung des magnesiumhaltigen Chlorophylls wird dabei aus Kohlendioxyd und Wasser unter Aufnahme von Lichtenergie Stärke gebildet. Dies geschieht durch einen Reduktionsprozess, in dessen Verlauf Sauerstoff frei gemacht wird.

Quantitativ wird der Energieumsatz bei der Kohlehydratbildung von folgender Gleichung wiedergegeben:



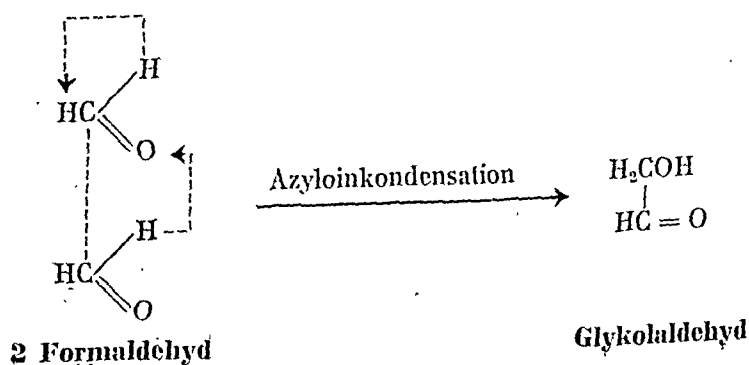
Die optimale Kohlensäureassimilation geht bei einer Lichtabsorption vonstatten, die dem Wellenlängengebiet 5700–6100 Ångström-Einheiten entspricht, in dem orangefarbenen Teil des Spektrums. In diesem Wellenlängengebiet liegt die maximale Lichtabsorption des Chlorophylls. Die absorbierte Lichtenergie löst die Stärkebildung aus, und die Kohlensäureassimilation ist um so intensiver, je stärker die Lichtabsorption ist.

Die Stärkebildung aus Kohlendioxyd und Wasser muss über eine Serie von Zwischenprodukten erfolgen. Diese sind nicht mit Sicherheit bekannt, aber schon *Baeyer* (1870) hielt das Formaldehyd für die erste Zwischenstufe

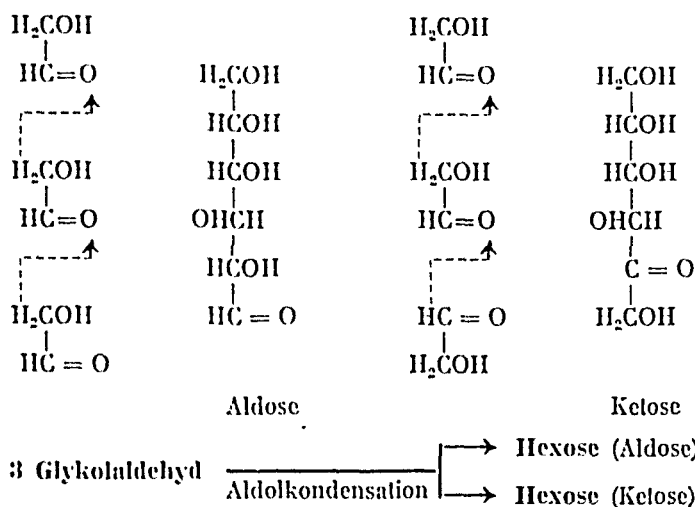


*Curtius* und *Franzen* haben auch in grünen Pflanzenteilen Spuren von Formaldehyd sowie kleine Mengen von flüchtigen Säuren, Aldehyden und ungesättigten Alkoholen nachgewiesen, womöglich mit der Hexosebildung aus Formaldehyd zusammenhängende Produkte. *Bokorny* konnte bei gewissen Grünalgen eine Stärkebildung in 0,001%iger Formaldehydlösung bereits im Dunkel feststellen. Bei einer Konzentration von 0,005 % wirkte die Formaldehydlösung dagegen giftig.

Man glaubt, dass die weitere Hexosebildung durch eine Reihe von Kondensations- und möglicherweise Reduktionsprozessen stattfindet, aber der Verlauf im einzelnen ist nicht mit Sicherheit bekannt. Es ist wahrscheinlich, dass zwei Formaldehydmoleküle durch eine Azyloinkondensation zunächst eine Diose, den Glykolaldehyd, bilden.



Durch fortgesetzte Aldolkondensation können sich dann drei Glykolaldehydmoleküle zu einer Hexose zusammenschliessen. Diese wird eine Aldose oder Ketose, je nach Orientierung des dritten Diosemoleküls.



Die Formaldehydhypothese der Stärkebildung ist in letzter Zeit angezweifelt worden (*Krebs* und *Eggleston* u. a.). Unter dem Einfluss des Lichts und durch Vermittlung der Chloroplasten scheint die Kohlensäure direkt an eine bisher unbekannte Substanz angelagert werden zu können, aus welcher dann Hexosemoleküle direkt abgespalten werden. Einzelheiten des Vorgangs sind noch nicht bekannt.

Die Kohlensäureassimilation der Pflanzen ist ein für den Kreislauf des Kohlenstoffs in der Natur fundamentaler Prozess. In den Pflanzenzellen werden die Hexosemoleküle unter Wasseraustritt polymerisiert, in erster Linie zu Dihexosemolekülen. Diese vereinigen sich dann zu höhermolekularen Polysacchariden, Stärke, Inulin usw., welche als Reservenernährung der Pflanze gespeichert werden.

Die im Zellprotoplasma entstehenden Stärkekörnchen sind nicht homogen, sondern enthalten neben eingelagertem Eiweiss noch Phosphorsäure. Sie haben einen geschichteten Bau. In der Hüllensubstanz ist das Polysaccharid an Phosphor-

säure gebunden, als *Amylopektin*, welches das reine Polysaccharid, die *Amylose*, mantelförmig umschliesst. Wenn die Stärke mit Alkali behandelt wird, kann die Phosphorsäure entfernt werden. Es entsteht da eine wasserlösliche, nicht kleisterbildende Stärke, die als *Zulkowskysche* lösliche Stärke bekannt ist.

Die Energie, welche bei Kohlensäureassimilation und Stärkebildung gebunden wird, wird bei Verbrennung der Stärke wieder frei gemacht. *Warburg* und *Negelein* haben bei der Verbrennung ca. 70 % der Strahlungsenergie wiedergefunden, welche während der Kohlensäureassimilation absorbiert worden war. Diese Energie wird bei Auflösung der Atombindungen frei. Dabei entspricht nach *Päuling* die Lösung einer

C—C	Bindung	ca.	58	kal
C—H	»	»	87	»
C=C	»	»	100	»
C≡C	»	»	123	»

## Umsetzung der Kohlehydrate im Tierorganismus

Der Aufbau des Glykogens im Tierorganismus geht wahrscheinlich analog mit dem der Stärke bei den Pflanzen vonstatten. Dabei kann jedoch die Lichtenergie nicht direkt ausgenützt werden. Die zur Glykogenbildung notwendige Energie wird statt dessen beim oxydativen Abbau von Hexosemolekülen frei.

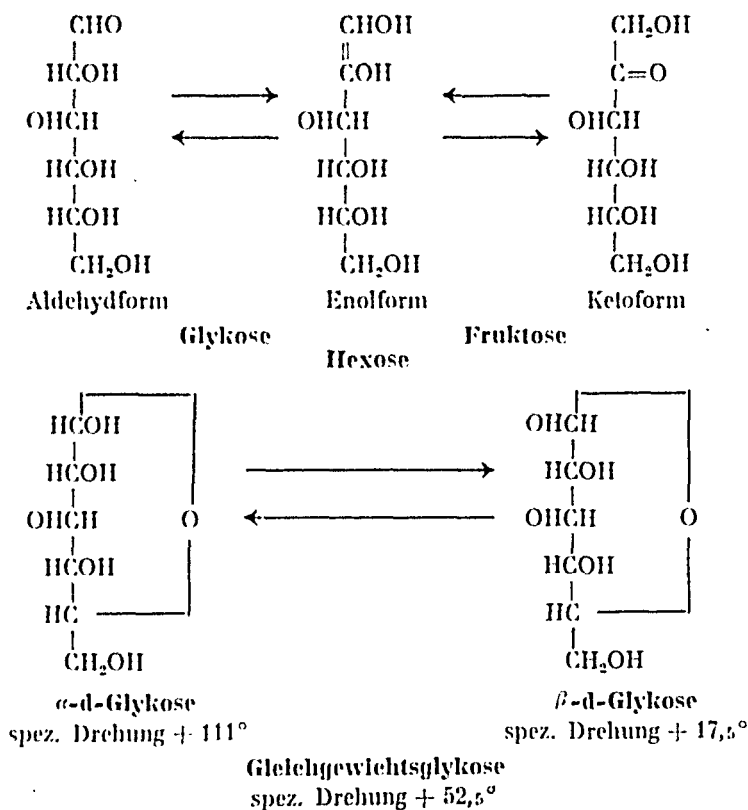
Unter dem Einfluss der Amylase zerfällt die Stärke in Maltose, ein Disaccharid, welches durch die Wirkung der Maltase in zwei Glykosemoleküle weitergespalten wird. Auch das Glykogen baut sich aus Glykose auf, aber das Glykogenmolekül zerfällt nicht in ein Disaccharid, sondern direkt in Glykose. Durch die Wirkung von Phosphorsäure erfolgt eine Auflösung des Glykogens, eine Phosphorolyse, bei der Hexosephosphorsäureester gebildet werden, ein für den Kohlehydratabbau im Organismus äusserst bedeutungsvoller Prozess (vgl. S. 100).

Die Glykose ist mithin der Grundstoff des Glykogens und

eine Substanz, welche in wechselnder Form auftreten kann. Sie kann zu hochmolekularen Polysacchariden polymerisiert werden. Bei den biochemischen Stoffwechselprozessen wird die Hexose normalerweise so weit abgebaut, dass Kohlendioxyd und Wasser entstehen.

### Umwandlungsformen der Glykose

Glykose oder Traubenzucker ist eine Aldose. Diese kann in einer mehr reaktiven *Enolform* auftreten, welche durch eine tautomere Verschiebung eines Wasserstoffatoms zur Aldehydgruppe der Glykose zustande kommt. Dabei entsteht eine Doppelbindung zwischen erstem und zweitem Kohlenstoffatom. Diese Enolform ist, wie man annimmt, die Veresterungsform der Hexose und gemeinsam für Glykose, Fruktose und Mannose.

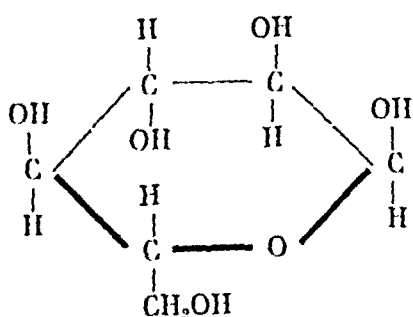




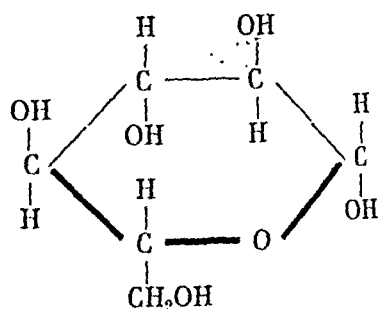
Innerhalb des Glykosemoleküls finden leicht intramolekulare Umlagerungen statt, am ehesten ein Ringschluss zwischen erstem und fünftem Kohlenstoffatom unter Vermittlung eines Sauerstoffatoms. Die Glykose tritt da in ihrer Laktonform als  $\alpha$ - und  $\beta$ -Glykose auf.

$\alpha$ - und  $\beta$ -Glykose sind beide rechtsdrehend, haben aber ein verschiedenes optisches Drehungsvermögen. In wässriger Lösung tritt mit der Zeit ein Gleichgewichtszustand zwischen  $\alpha$ - und  $\beta$ -Glykose ein, wobei sich die optische Drehung der Glykoselösung zu einer konstanten Mittellage ändert; das Phänomen wird als *Mutarotation* bezeichnet.

Durch Ringbildung aus 5 Kohlenstoffatomen und einem Sauerstoffatom entsteht ein *Pyran*kern. Nach *Haworth* ist daher die Laktonform der Glykose eine  $\alpha$ - oder  $\beta$ -Glykopyranose.

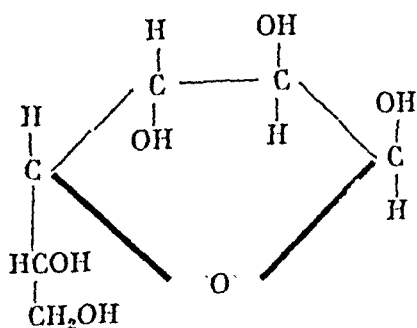


$\alpha$ -Glykopyranose

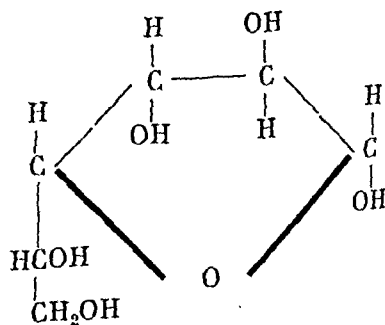


$\beta$ -Glykopyranose

Eine Ringbildung kann auch dadurch zustande kommen, dass das Sauerstoffatom das erste und vierte Kohlenstoffatom verbindet. Dabei entsteht die mehr reaktive  $\gamma$ -Form. Eine



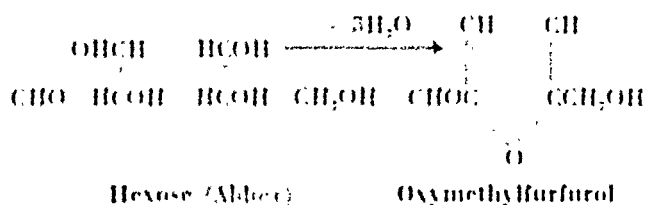
$\alpha$ -Glykofuranose



$\beta$ -Glykofuranose

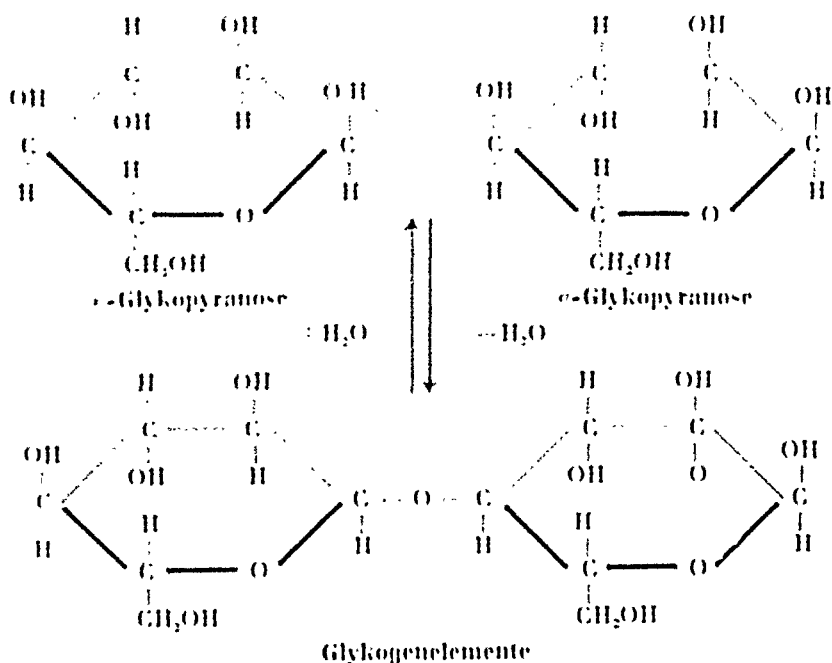
Ringbildung aus 4 Kohlenstoffatomen und einem Sauerstoffatom führt zu einem *Furankern*. Die  $\gamma$ -Glykose ist demzufolge nach *Haworth* eine  $\alpha$ - oder  $\beta$ -Glykofuranose.

Werden Hexosen mit Mineralsäuren erwärmt, so wird denselben Wasser entzogen, und die Hexosen gehen in Oxymethylfurfural über.



Das Oxymethylfurfural gibt mit Resorzin oder Orzin stark gefärbte Verbindungen, welche zu spezifischen Hexosereaktionen verwertet werden können (vgl. S. 287).

### Bildung und Abbau des Glykogens



Das Glykogen entsteht durch eine Anhydridbildung, wobei sich Hexosemoleküle unter Wasseraustritt zu grösseren Molekülaggagaten zusammenschliessen. Dabei werden die einzelnen Hexosemoleküle von Sauerstoffbrücken zu zweit unter Bildung von Disacchariden zusammengehalten, welche fernerhin zu Polysacchariden vom Typ der Stärke und des Glykogens polymerisiert werden.

Das Glykogen ist nach *Karrer* eine polymerisierte Diamylose, die ihrem Bau nach mit dem Amylopektin übereinstimmt, aber mit anderem Polymerisationsgrad. Bei seinem Zerfall lässt das Leberglykogen den normalerweise im Organismus vorhandenen Blutzucker entstehen, der für eine  $\alpha$ -Glykopyranose gehalten wird.

Der Blutzucker wird in der Muskulatur sekundär zu Muskelglykogen resynthetisiert. Im ruhenden Muskel wird dieses Glykogen langsam verbraucht, bei Muskelarbeit dagegen rascher.

Der tätige Muskel bekommt eine saure Reaktion. *Hopkins* und *Fletcher* haben zuerst nachgewiesen, dass während der Arbeitsperiode des Muskels Milchsäure gebildet wird. In der Ruheperiode wird dagegen wieder Muskelglykogen erzeugt.

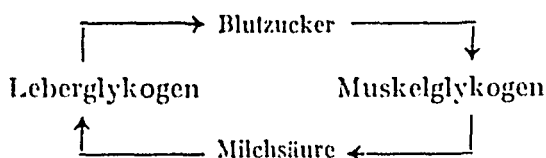
Die Umsetzung der Kohlehydrate in der Muskulatur verläuft während zweier verschiedener Phasen, der *anaeroben*, welche zur Milchsäurebildung führt, und der *aeroben*, in der die Milchsäure verschwindet. Durch eine Serie von gekoppelten Oxydo-Reduktionsprozessen im Organismus wird die Milchsäure während der *aeroben* oder *oxydativen* Phase zum Teil wieder in Glykogen umgewandelt, auf Kosten eines anderen Teils der Milchsäure, welcher zu Kohlendioxyd und Wasser verbrannt wird.

Nach *Meyerhof* stehen Verschwinden des Muskelglykogens während der anaeroben und Wiederbildung desselben während der oxydativen Phase in einem gesetzmässigen Verhältnis zueinander. Aus der beim anaeroben Abbau des Muskelglykogens gebildeten Milchsäure wird während der oxydativen Phase zum Teil Glykogen resynthetisiert. Hierbei entsteht nach *Meyerhof* wieder Glykogen aus vier Milchsäuremolekülen auf Kosten eines fünften, das zu Kohlendioxyd und Wasser ver-

brannt wird, wobei Energie zur Resynthese des Glykogens frei gemacht wird. Diese *Meyerhofsche* Theorie schien seinerzeit eine formale Erklärung für die Reaktionskoppelung beim Abbau des Glykogens und der Neubildung desselben aus Milchsäure zu geben. Die experimentellen Unterlagen der Theorie erscheinen indessen durch Arbeiten von *Wieland* u. a., durch welche die Richtigkeit des sog. *Meyerhofschen* Quotienten in Zweifel gezogen wurde, in einem anderen Licht.

Wird die Milchsäure nicht in der Muskulatur verbrannt oder dort in Glykogen zurückverwandelt, so geht sie in das Blut über. Daher steigt der Milchsäuregehalt des Blutes bei Muskelarbeit. Die Milchsäure wird in der Leber zu Glykogen umgebildet, von wo sie dann in Form von Blutzucker von neuem der Muskulatur zugute kommt. Bei intensiver Muskelarbeit wird der Organismus mit Milchsäure überschwemmt. Findet die Glykogensynthese in der Leber nicht schnell genug statt, so wird die Milchsäure im Urin ausgeschieden (*Colasanti* und *Moscatelli*).

*Muskulatur und Leber stehen folglich beim Kohlehydratumsatz in enger Beziehung zueinander. Das System bildet einen inneren Kreislauf der Kohlehydrate.*



Energiegebende Nahrungsstoffe, Fette, Kohlehydrate und Eiweisskörper, welche dem Organismus als Speise zugeführt werden, werden teilweise in Glykogen umgewandelt. Das Glykogen kann dann bei seinem Zerfall durch eine Serie von Zwischenstufen in die Endprodukte der normalen Verbrennung, Kohlendioxyd und Wasser, übergehen. Beim Abbau von Kohlehydraten im Organismus ist eine Mitwirkung von Phosphorsäure im allgemeinen eine notwendige Voraussetzung. *Needham* hat jedoch bei Hühnerembryonen einen Glykoseabbau beobachtet, bei welchem phosphorylierte Zwischenstufen nicht nachgewiesen werden konnten.

## Der Phosphorylierungsprozess

Die biologische Bedeutung der Phosphorsäure für den Abbau von Kohlehydraten ist erst durch das Studium der alkoholischen Gärung bekannt geworden.

*Harden* und *Young* fanden 1905, dass die Anwesenheit von Phosphorsäure für den normalen Gärungsverlauf erforderlich ist. Beim Abbau des Hexosemoleküls muss dasselbe mit Phosphorsäure zu Hexosephosphorsäureestern gekoppelt werden. Die zuerst gefundene Hexosephosphorsäure war ein Hexose-1,6-diphosphorsäureester. *Robison* fand 1914 ausserdem in geringerer Menge eine Hexose-6-monophosphorsäure.

Wird der Phosphorylierungsprozess gestört, dann wird das Hexosemolekül im Organismus nicht mehr normal abgebaut. Dabei entstehen Störungen des normalen Stoffwechsels mit Anhäufung von Glykose bzw. intermediären Umsetzungsprodukten. Wenn diese nicht weiter umgewandelt werden können, müssen sie aus dem Organismus ausgeschieden werden. Der Energieumsatz nimmt dabei ab. Wird er ungenügend, so kommen die Lebensvorgänge infolge Energiemangels völlig zum Stehen, und der Tod tritt dann aus diesem Grunde ein, wenn er nicht schon früher durch Vergiftung mit toxischen Abbauprodukten hervorgerufen wurde. Die Phosphorylierung der Hexosemoleküle ist somit ein lebenswichtiger Prozess, und eine nähere Kenntnis des Verlaufs ist deshalb von Bedeutung.

## Die zellfreie Gärung

*Buchner* hatte 1897 nachgewiesen, dass eine normale Alkoholgärung stattfinden konnte, auch wenn die Hefezellen mechanisch zerstört wurden. Der zellfreie Presssaft enthält ein Fermentsystem, die *Zymase*, welches imstande ist, Glykose auch ohne die direkte Mitwirkung der Hefezellen in Alkohol umzuwandeln. Dieser zellfreie Presssaft lässt sich in zwei Komponenten teilen, eine dialysierbare, thermostabile, eiweissfreie, von *v. Euler* und *Myrbäck* später als *Cozymase* bezeichnete, und eine nicht dialysierbare, thermolabile, die *Apozymase*,

welche wirksame Fermentproteine enthält. Das komplette Ferment enthält die Cozymase in einer reversibel dissozzierbaren Verbindung mit seinen Fermentproteinen.

Es ist noch ungeklärt, ob die Cozymase direkt bei der Phosphorylierung der Glykosemoleküle mitwirkt, oder ob die Wirkung derselben hierbei nur eine indirekte ist, durch Eingreifen der Cozymase in den Abbauprozess im übrigen (vgl. S. 171).

Zur Phosphorylierung muss die Hexose zunächst in eine mehr reaktive Veresterungsform umgelagert werden. Für die letztere hält man die der Glykose, Lävulose und Mannose gemeinsame Enolform.

In dem in Tätigkeit befindlichen Gärungssystem besteht ein Gleichgewichtszustand der Veresterungsform einerseits mit der ursprünglichen Glykose und andererseits mit der entstandenen Hexosemonophosphorsäure.

Glykose  $\rightleftharpoons$  Veresterungsform  $\rightleftharpoons$  Hexosemonophosphorsäure  $\longrightarrow$  Abbau

Bei Abwesenheit von Cozymase bleibt die Phosphorylierung der Hexose aus. Auch die Bildung der Hexosemonophosphorsäure unterbleibt, wenn der weitere Abbauprozess verhindert wird.

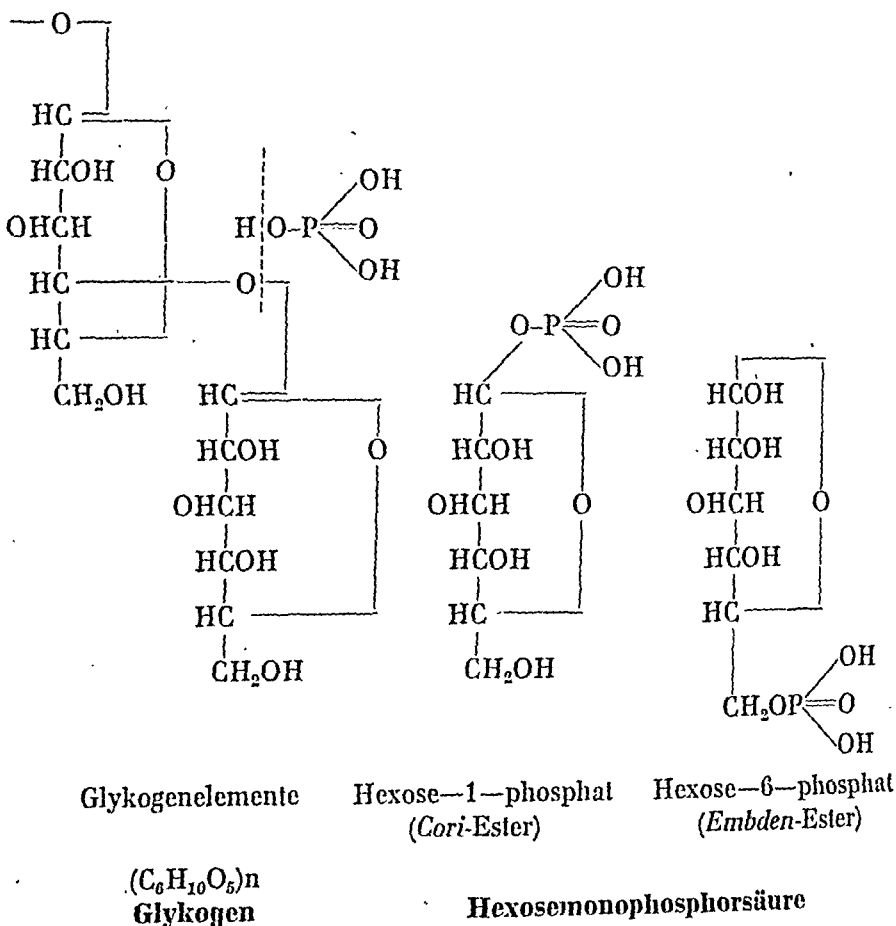
Im Gegensatz hierzu zeigte *Ragnar Nilsson* 1929, dass *Glykogen* in einem System, in dem die totale Glykolyse durch Fluoridvergiftung gehemmt ist, nach wie vor in Hexosemonophosphorsäure umgewandelt werden kann, und zwar ohne Mitwirkung von Cozymase.

Glykogen  $\rightleftharpoons$  Hexosemonophosphorsäure  $\longrightarrow$  Abbau

Der Glykogenzerfall ist daher nicht an den Abbau der Hexosemonophosphorsäure gekoppelt. Zwischen Glykogen und Hexosemonophosphorsäure tritt ein Gleichgewichtszustand ein, in welchem entsprechend der Bildung und dem Abbau des Glykogens Schwankungen zustande kommen. Bei starkem Glykogenübergewicht schreitet der Phosphorylierungsprozess praktisch kontinuierlich nur in einer Richtung weiter. *Es liegt folglich ein grundsätzlicher Unterschied zwischen Glykose und Glykogen hinsichtlich der Bildung der Hexosemonophosphorsäure vor.*

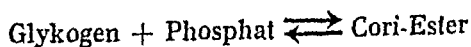
## Phosphorolyse des Glykogens.

*Parnas* nimmt an, dass das Glykogen durch den Einfluss von Phosphorsäuremolekülen zerfällt, welche die Glykosidbindungen in den Glykogenelementen auflösen, wobei Hexosemonophosphorsäure direkt durch sukzessive Abspaltung aus dem Glykogen gebildet wird.



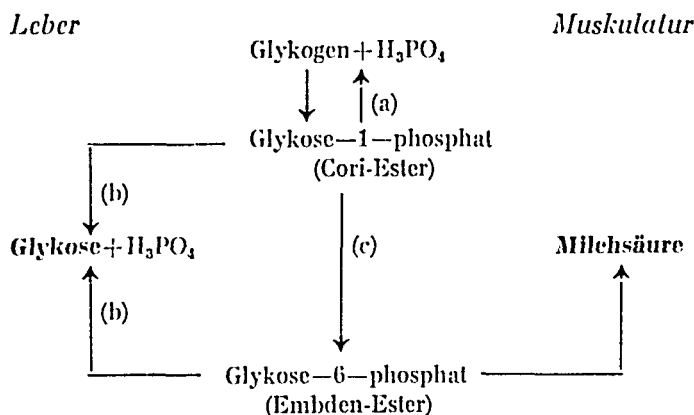
*Cori*, *Colowick* und *Cori* haben nachgewiesen, dass bei Phosphorolyse des Glykogens intermediär Glykose-1-phosphat entsteht, der labile *Cori-Ester*, welches dann unter dem Einfluss eines Enzyms, der Phosphoglykomutase, zu Glykose-6-phosphat, dem beständigeren *Emden-Ester*, umgelagert wird.

*Kiessling* hat aus Hefe eine Eiweissfraktion, das G-Protein, dargestellt, welches die reversible Reaktion



katalytisch beeinflusst. Diese C-Protein-Fraktion lässt die Phosphoglykomutasewirkung vermissen. Durch fraktionierte Fällung mit Ammoniumsulfat konnte *Kiessling* das C-Protein in zwei Fraktionen spalten. Die eine Fraktion hatte phosphorylierende Wirkung und förderte die Entstehung des *Cori*-Esters. Die andere Eiweissfraktion wirkte dephosphorylierend und zerteilte wieder die gebildete Hexosemonophosphorsäure in Glykose und freie Phosphorsäure. Unabhängig von der Glykogenmenge tritt daher durch das Zusammenwirken der beiden C-Protein-Fraktionen ein Gleichgewichtszustand zwischen *Cori*-Ester und anorganischem Phosphat ein.

*Warburg* und seine Mitarbeiter haben ebenfalls aus Hefe zwei spezifische Eiweisskörper isoliert, Protein A und Protein B. Nach *Meyerhof*, *Kiessling* und *Schulz* wirkt das Protein A katalytisch auf die Freimachung der Phosphorsäure aus der Phosphobrenztraubensäure ein, das Protein B weist dagegen die Phosphoglykomutasewirkung auf, welche den *Cori*-Ester in den *Embden*-Ester umwandelt. Magnesium- und Manganionen fungieren bei dieser Phosphoglykomutasewirkung als Aktivatoren. Hier folgt eine schematische Darstellung der Phosphorolyse des Glykogens nach *Cori*, *Cori* und *Schmidt*.



(a) Phosphorylasewirkung.

(b) Phosphatasewirkung.

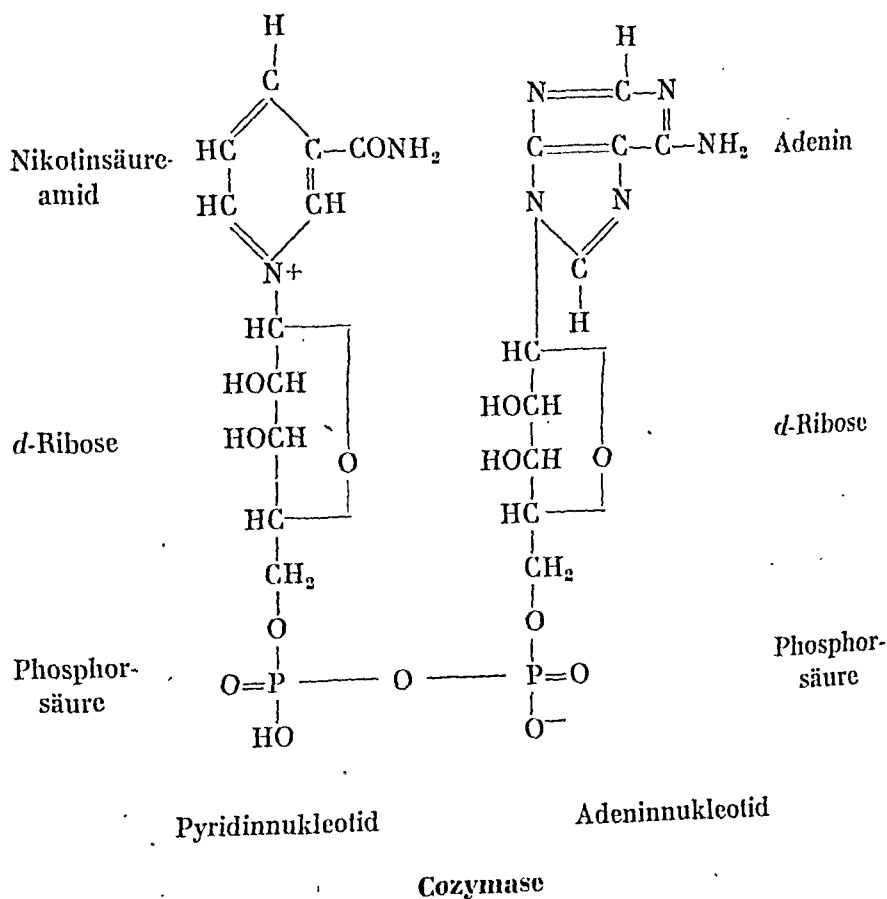
(c) Phosphoglykomutasewirkung. Diese wird von Mangan- und Magnesiumionen katalytisch beeinflusst.



## Phosphorylierung der Glykose

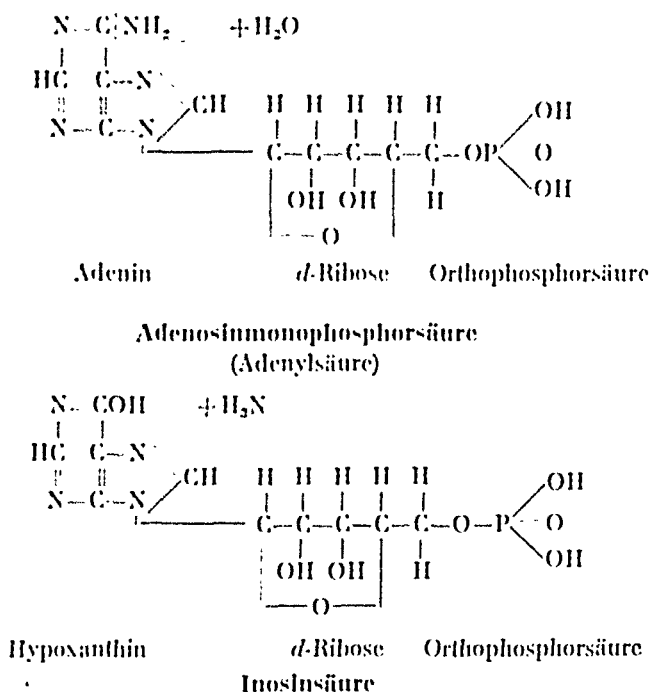
Im Gegensatz zum Glykogen erfordert die Glykose zu ihrer Phosphorylierung die Beteiligung von Cozymase. Der Bau der letzteren ist heute durch Arbeiten von *Warburg* und *Christian*, *Euler*, *Myrbäck*, *Karrer*, *Levene*, *Schlenk*, *Lohmann* u. a. weitgehend, wenn auch nicht endgültig, geklärt. Die Cozymase ist ein Pyridinnukleotid, dass sich aus Adenin, zwei Phosphorsäure- und zwei Pentosemolekülen (*d*-Ribose) sowie Nikotinsäureamid zusammensetzt. Durch Reduktion und Nuklease-wirkung zerfällt die Cozymase auf die im V. Abschnitt näher behandelte Weise in ein Adeninnukleotid und ein Pyridinnukleotid.

Der Bau der Cozymase ist nach *Schlenk*



## Das Adenylsäuresystem

Das Adeninnukleotid, die Adenosinmonophosphorsäure, welches beim Zerfall der Cozymase entsteht, ist seinem Bau nach mit der *Muskeladenylsäure* identisch. Diese setzt sich aus Adenin, *d*-Ribose und Orthophosphorsäure zusammen. Durch hydrolytische Abspaltung von Ammoniak entsteht die *Inosinsäure*, ein Monophosphornukleotid, welche schon *Liebig* unter den phosphorhaltigen organischen Substanzen der Muskulatur gefunden hatte. Diese enthält das Oxypurin Hypoxanthin an Stelle von Adenin.



## Adenylsäure und Ammoniakbildung

*Emden* (1928) und *Parnas* haben eine Ammoniakbildung in der Muskulatur bei Muskelkontraktionen nachgewiesen. Diese Ammoniakbildung findet während der anaeroben Phase

durch eine Desaminierung der Muskeladenylsäure statt. Dabei entsteht unter Mitwirkung einer spezifisch wirkenden Adenylsäuredeaminase Inosinsäure. Während der oxydativen Phase im Ruhestadium des Muskels verschwindet das gebildete Ammoniak, wobei wieder Muskeladenylsäure aus Inosinsäure entsteht.

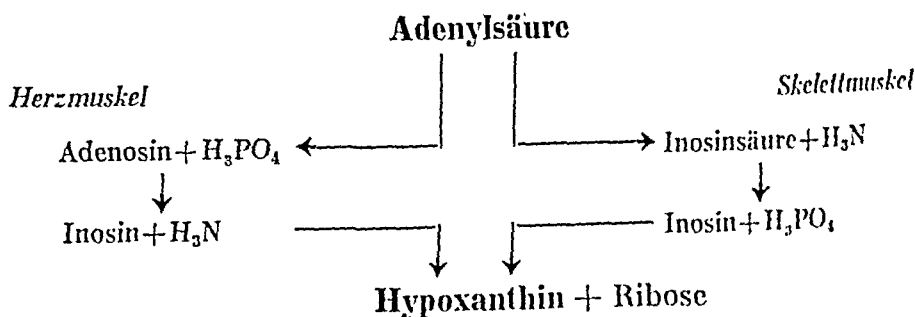


Nach *Parnas* (1928) ist bei dieser oxydativen Resynthese die Beteiligung einer katalytisch wirkenden Substanz erforderlich, welche die Überführung des Ammoniaks in Inosinsäure zwecks Wiederbildung der Adenylsäure vermittelt. Diese Substanz ist noch nicht näher bekannt.

Auch Phosphorsäure wird aus sowohl Adenylsäure wie Inosinsäure abgespalten, wobei Adenosin bzw. Inosin entsteht. Hierzu gehört die Mitwirkung von Phosphatase. Die Desaminierung der Adenylsäure scheint unabhängig von der Dephosphorylierung erfolgen zu können, jedoch wird in gewissen Fällen der Desaminierungsprozess bei Überschuss von Phosphat gehemmt.

Nach *Lutwak-Mann* (1936) verläuft die Desaminierung in Herz- und Skelettmuskulatur verschieden.

Den Abbau der Adenylsäure in der Muskulatur veranschaulicht das folgende Schema nach *Lutwak-Mann*.

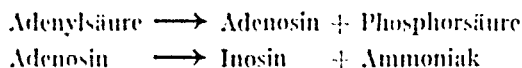


*Lutwak-Mann* hat Hypoxanthin beim Abbau des Adenosins durch *Bact. coli* isoliert, konnte aber da die Ribose nicht wiederfinden, welche wahrscheinlich weiterumgesetzt wird.

Durch die Wirkung von Xanthinoxydase (s. S. 169) kann das Hypoxanthin zu Harnsäure weiteroxydiert werden. *Hier gibt es einen Weg für die endogene Harnsäurebildung aus Adenylsäure.*

Adenylsäure kommt nicht nur in Muskelzellen vor, sondern in allen lebenden Zellen. Adenylsäure-desaminase ist ebenfalls nach *Conway* und *Cooke* in tierischen Organen und Geweben verbreitet, am reichlichsten jedoch in der Skelettmuskulatur der Säugetiere.

Schon im primitiven Ei findet sich Adenylsäure-desaminase. *Örström* (1935) hat gezeigt, dass die Ammoniakbildung im Seeigeei nach der Befruchtung erheblich zunimmt. Diese Ammoniakbildung kommt durch Desaminierung von Adenylsäure und Adenosin zustande. Sie scheint analog dem Vorgang in der Herzmuskulatur und nach folgendem Schema zu verlaufen:



*Weil-Malherbe* (1938) hat gefunden, dass die Anwesenheit von Ammoniumchlorid die zelluläre Atmung steigert, die anaerobe Glykolyse hemmt und die aerobe Phase des Glykogenabbaus begünstigt. Dies war namentlich bei Präparaten von Gehirn und Milz des Meerschweinchens der Fall. Die Atmung des Nierengewebes kann durch Zusatz von Ammoniumchlorid mit Glykose oder Laktat als Nährsubstanz um 50 % gesteigert werden. Das Ammoniak scheint hierbei eine katalytische Rolle zu spielen, da die zugesetzte Ammoniummenge unverändert wiedergefunden wird.

*Örström* (1940) konnte ebenfalls nachweisen, dass Ammoniak und Aminosäuren den Atmungsprozess und den Abbau des Glykogens im unbefruchteten Seeigeei steigern. Der biologische Oxydationsprozess ist für den normalen Kohlehydratumsatz ausschlaggebend. Durch die bei der Oxydation der kohlehydrate frei gemachte Energie wird aus der Inosinsäure von neuem Adenylsäure gebildet. Dabei verschwindet das Ammoniak, und die oxydationssteigernde Wirkung desselben fällt weg. Hierdurch wird der oxydative Abbau eingeschränkt.

*In dieser Weise tritt ein Gleichgewichtszustand ein, der den Verlauf des biochemischen Kohlehydratabbaus in der lebenden Zelle steuert, mit Ammoniak und Adenylsäure als regulatorisches System.*

Die oxydationsfördernde Wirkung des Ammoniaks ist wahrscheinlich die Ursache des spezifisch dynamischen Effekts eiweissreicher Nahrung beim Stoffwechsel (vgl. S. 369).

Krebs (1932) hat gezeigt, dass die Atmung von Nierenschnitten nicht nur dann von Ammoniumchlorid gesteigert wird, wenn Glykose vorhanden ist, sondern auch bei Anwesenheit von Ketosäuren als Nährstoff. Hierbei scheint sich jedoch das Ammoniak irgendwie an der Reaktion zu beteiligen, da dasselbe als Ammonium verschwindet, aber als Aminosäuren wieder zum Vorschein kommt. Das Verschwinden der Ketonkörper ist in diesem Falle mit einem Aminierungsprozess mit Bildung von Aminosäuren verbunden.

Beim Diabetes findet man nicht selten eine Diskrepanz, wenn die Bestimmung des ausgeschiedenen Ammoniakstickstoffs durch Überdestillieren von freiem Ammoniak erfolgt, oder wenn die Bestimmung durch Formoltitrierung vorgenommen wird, welche auch für Aminosäuren einen Ausschlag gibt.

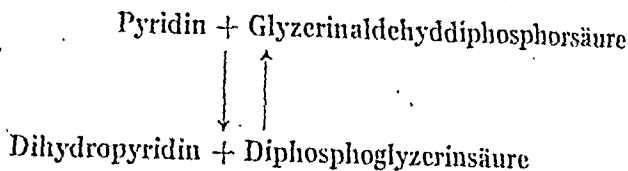
### Adenylsäure und Phosphorylierungsprozess

Die Adenylsäure besitzt noch eine biologisch wichtige Eigenschaft: sie kann Phosphorsäure übernehmen, z. B. aus der Phosphobrenztraubensäure, welche intermediär beim Abbau der Glykose gebildet wird (vgl. S. 119). Dabei entsteht Brenztraubensäure unter gleichzeitiger Bildung von Adenosintriphosphorsäure.

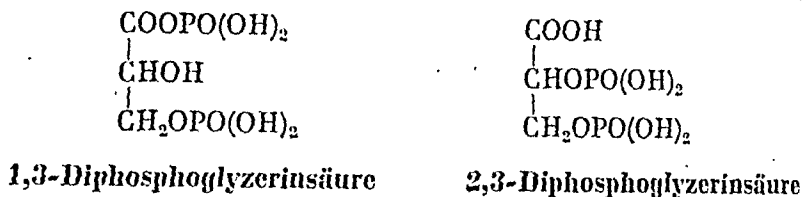
Durch die Mitwirkung von Pyridinnukleotiden gelegentlich des biologischen Oxydationsprozesses (vgl. S. 170) kann die aufgenommene Phosphorsäure an andere Substanzen gekoppelt werden. Es entsteht da wiederum Adenosinmonophosphorsäure, worauf sich der Vorgang durch Aufnahme neuer Phosphorsäure wiederholen kann.

Die Adenylsäure gewinnt dadurch grosse Bedeutung für den Phosphorylierungsprozess und wirkt bei der Überführung der





Nach *Negelein* und *Brömel* handelt es sich um eine 1,3-Diphosphoglyzerinsäure.



Diese 1,3-Diphosphoglyzerinsäure ist labil und von der von *Greenwald* entdeckten 2,3-Diphosphoglyzerinsäure verschieden, welche stabiler ist und ihre Phosphorsäure nicht so leicht abgibt.

Die gebildete 1,3-Diphosphoglyzerinsäure zerfällt hydrolytisch in Phosphorsäure und Phosphoglyzerinsäure, welche weiter in Phosphobrenztraubensäure übergeht (vgl. S. 119).

Die frei gemachte Phosphorsäure wird von Adenylsäure unter Bildung von Adenosintriphosphorsäure aufgenommen. Da das *Glyzerinaldehydphosphat* anorganische Phosphorsäure zur Bildung des Diphosphats direkt anlagern kann, ist die Zufuhr von anorganischer Phosphorsäure zum Adenylsystem auf diesem Wege möglich (vgl. S. 299).

2. Aus der beim Kohlehydratabbau entstandenen *Phosphobrenztraubensäure* wird durch hydrolytische Dissoziation freie Phosphorsäure abgespaltet, wobei sich *Brenztraubensäure* bildet (vgl. S. 119). Die frei gemachte Phosphorsäure wird von der Adenylsäure direkt aufgenommen, wobei die Adenosintriphosphorsäure wiedergebildet wird.

Durch den Kohlehydratabbau wird also dem Adenylsystem beständig Phosphorsäure zugeführt, und die Adenosintriphosphorsäure gibt ihrerseits Phosphorsäure für die Phosphorylierung des Hexosemoleküls ab. Auf diese Weise kommt

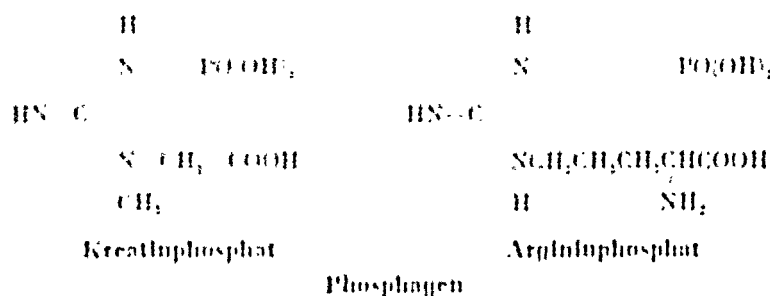
es zu einem ständigen Kreislauf des Phosphorsäuremoleküls beim Kohlehydratumsatz.

3. Aus Phosphagen kann bei der Muskelarbeit auch Phosphorsäure zu dem Adenylsäuresystem geliefert werden.

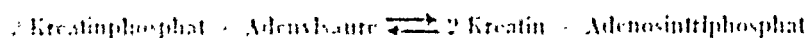
### Das Phosphagen-Kreatinsystem

Beim Phosphorylierungsprozess in der Muskulatur spielt das Kreatin eine wichtige Rolle.

Schon *Liebig* hatte festgestellt, dass der Gehalt des Muskels an Kreatin und auch anorganischem Phosphat bei Arbeit zunahm. Es muss daher eine bei der Muskelarbeit wirksame Substanz vorhanden sein, welche bei ihrem Zerfall Kreatin und Phosphorsäure entstehen lässt. Diese Substanz wurde 1926 von *Lepple* entdeckt und im folgenden Jahre von *Fiske* und *Subbotin* als Kreatinphosphat identifiziert. In der Muskulatur der wirbellosen Tiere ist die Phosphorsäure statt dessen an Arginin als Argininphosphat gebunden. Kreatinphosphat und Argininphosphat werden als *Phosphagen* zusammengefasst.



Bei Anwesenheit von Adenylsäure zerfällt nach *Lohmann* das Phosphagen in Kreatin bzw. Arginin und Phosphorsäure:



Beim Kohlehydratumsatz im Zusammenhang mit der Muskelarbeit wird daher das Phosphagen zu einer wichtigen Phosphorsäurequelle für das Adenylsystem.





## Der biochemische Abbauprozess der Hexose

Die Bildung der Hexosemonophosphorsäure leitet den Kohlehydratabbau im Organismus ein. Der gewöhnliche, Energie frei machende Hexoseabbau erfolgt durch eine initiale oxydo-reduktive Spaltung des Hexosemoleküls mit Entstehung zweier Triosen. Dieser *Hexo-Triosenabbau* verläuft teils in einer anaeroben Phase, welche ohne Mitwirkung von Sauerstoff weitergeht, und teils in einer oxydativen Phase, bei der die Beteiligung von Sauerstoff unerlässlich ist.

Neben diesem Hexo-Triosenabbau hat *Dickens* (1938) eine andere Form von oxydativer Spaltung der Hexosemonophosphorsäure entdeckt, welche nicht auf dem Wege über Triosen vor sich geht, sondern durch Dekarboxylierung mit Pentosebildung als erster Stufe. Dieser *Hexo-Pentosenabbau* ist zuerst im Seeigeli nachgewiesen worden und scheint u. a. in Zellen aufzutreten, welche sich im Wachstum oder Teilung befinden.

### 1. Der Hexo-Triosenabbau

Nach Bildung der Hexosemonophosphorsäure erfolgt bei diesem Abbau eine Oxydo-Reduktionsspaltung zwischen Kohlenstoffatom 3 und 4. Von den dabei entstehenden beiden Triosen ist die eine phosphoryliert. Erfolgt der Abbau aus Hexosediphosphorsäure, so sind beide gebildeten Triosen phosphoryliert.

Die Oxydo-Reduktionsspaltung und der eigentliche Abbauprozess finden durch eine Serie von intramolekularen Umlagerungen statt und erfordern nicht die Beteiligung von Sauerstoff. Dieser Teil des Abbauprozesses wird als die *anaerobe Phase* zusammengefasst. Diese kommt bei Bildung von intermediären Stoffwechselprodukten, welche nicht weiterverbrannt werden, z. B. Äthylalkohol und Milchsäure, zum Stehen. Zum vollständigen Abbau zu Kohlendioxyd und Wasser ist die Mitwirkung von Sauerstoff unerlässlich. Beim oxydativen Abbauprozess, der über eine Serie von Dehydrierungen und Dekarboxylierungen vonstatten geht, wird Energie frei gemacht, wel-

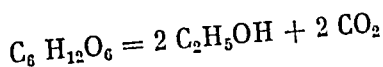
che den Energie erfordernden synthetischen Prozessen zugute kommen kann. Der oxydative, Energie frei machende Abbau-prozess wird zusammenfassend als die *aerobe* oder *oxydative Abbauphase* bezeichnet.

### Die anaerobe Abbauphase

Der anaerobe Abbauprozess geht in verschiedenen Zellsystemen nicht in gleicher Weise vonstatten und führt zu verschiedenen Intermediärprodukten, je nachdem, ob die Ferment-systeme der Zellen unversehrt oder geschädigt sind. Die Alkoholgärung der Hefezelle und die Milchsäurebildung in der Muskulatur sind zwei Beispiele von wichtigen, anaerob verlaufenden Abbauprozessen.

### Die Alkoholgärung

Bei der Alkoholgärung wird aus der Hexose unter Mitwirkung von Phosphorsäure Äthylalkohol und Kohlendioxyd gebildet, was gemäss der *Gay-Lussacschen* Reaktion



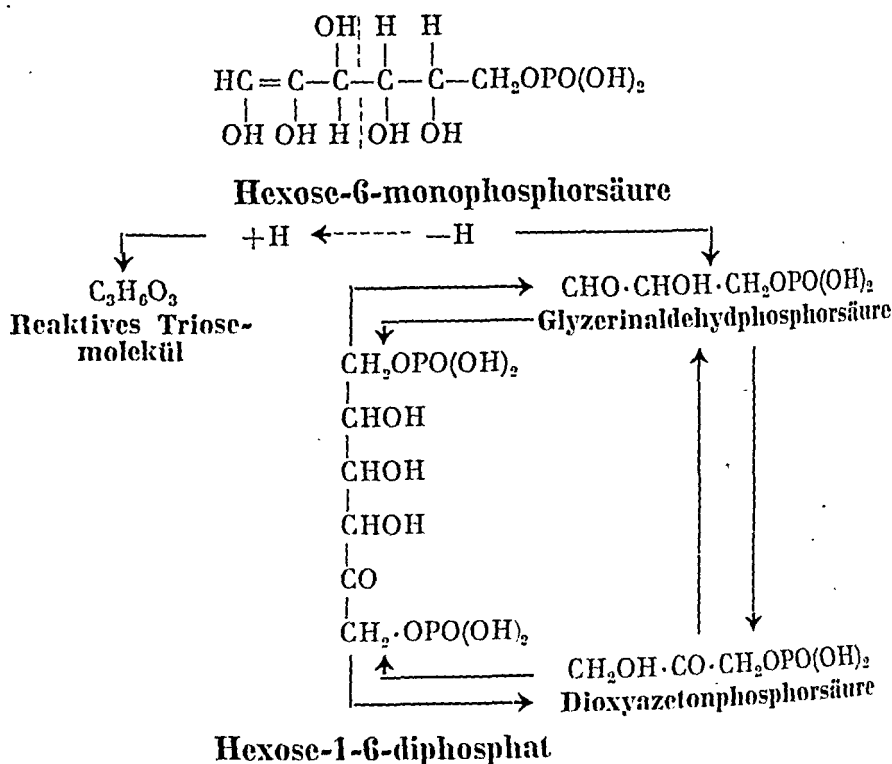
erfolgt.

Durch ein eingehendes Studium des Vorgangs in durch Fluorid gehemmten Gärungssystemen hat *Ragnar Nilsson* (1930) gefunden, dass das Hexosemolekül — nach Umlagerung in eine für Glykose, Lävulose und Mannose gemeinsame Vesterungsform (Enolform), sowie zu Hexosemonophosphorsäure phosphoryliert — zwischen Kohlenstoffatom 3 und 4 durch einen Oxydo-Reduktionsprozess gespalten wird. Die so entstandene Triose ist eine Glyzerinaldehydphosphorsäure. Bei Anwesenheit von Azetaldehyd wird die Glyzerinaldehydphosphorsäure zu Monophosphoglyzerinsäure oxydiert, wobei gleichzeitig der Azetaldehyd zu Äthylalkohol reduziert wird. Die Monophosphoglyzerinsäure wird weiter in Phosphorsäure und Brenztraubensäure gespalten. Durch Dekarboxylierung geht die Brenztraubensäure in Azetaldehyd und Kohlendioxyd



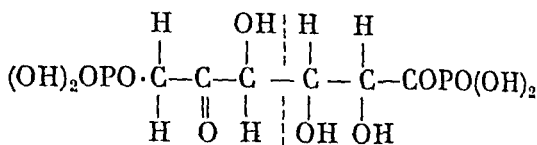
lich ist, dass es sich hier um einen ganz besonders reaktiven Körper handelt, der u. a. zu Brenztraubensäure glatt oxydiert wird. Der Reaktionsweg ist dabei allerdings noch nicht näher bekannt. Es ist in diesem Zusammenhang auf die Möglichkeit zur Resynthese von Hexosemolekülen durch eine Aldolkondensation, evtl. mit anschliessender Polysaccharidbildung, hingewiesen worden.

In einem destruierten Gärungssystem oder bei Abwesenheit von Azetaldehyd macht der Abbauprozess bei der Bildung von Glyzerinaldehydphosphorsäure halt. Letztere Substanz steht in enzymatischem Gleichgewicht mit Dioxyazetonphosphorsäure und dadurch schliesslich auch mit Hexose-1-6-diphosphorsäure. Es tritt ein Gleichgewichtszustand zwischen Triosephosphorsäure und Hexose-1-6-diphosphorsäure ein, worauf zuerst *Ragnar Nilsson* (1930), gestützt auf eigene Versuche, hingewiesen hat, und der später durch neue Versuche von *Barrenscheen* und *Beneschowsky* sowie *Meyerhof* und *Lohmann* experimentell gesichert wurde.

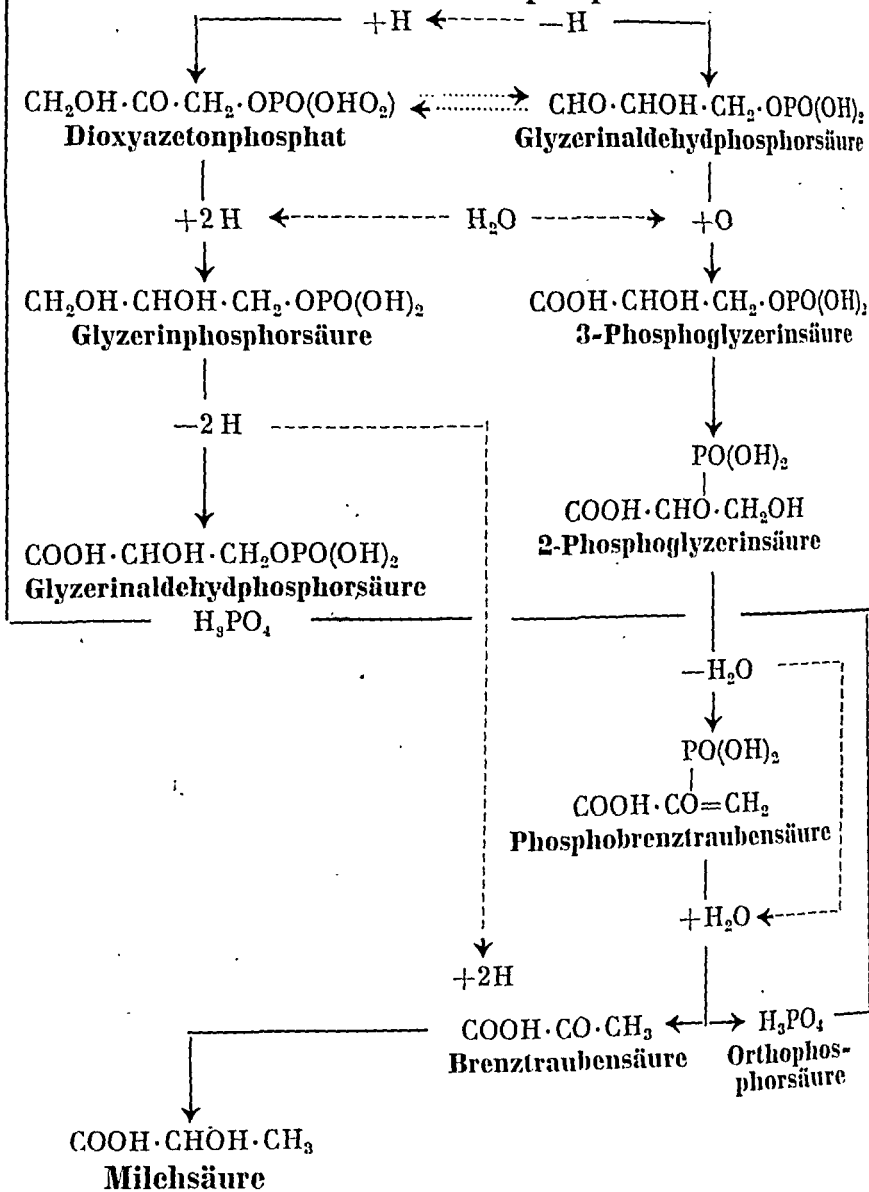




**Glyzerinaldehyd-  
diphosphorsäure**



**Hexose-1-6-diphosphorsäure**



bensäure gebildet, wahrscheinlich mit Methylglyoxal und Glycerinphosphorsäure als Zwischenstufen.

Soweit die Glycerinaldehydphosphorsäure nicht unmittelbar abgebaut wird, geht dieselbe in Hexose-1-6-diphosphorsäure über. Dies ist in grossem Umfang in der Muskulatur der Fall. In der Folge kann sich dann aus der Hexosediphosphorsäure Milchsäure bilden, aber der Umsetzungsprozess erhält dabei einen anderen Verlauf, der S. 118 schematisch dargestellt ist.

Nach *Meyerhof* wird auch hier der Abbauprozess von einer Oxydo-Reduktionsspaltung mit Bildung zweier Triosen eingeleitet, welche beide phosphoryliert sind. Nach *Emden* erfolgt der weitere Abbau über Dioxyazetonphosphat und Glycerinaldehydphosphat mit Glycerinphosphorsäure als Zwischenstufe. Das Glycerinaldehydphosphat kann nach *Warburg* und *Christian* temporär noch ein Phosphorsäuremolekül binden und durch Beteiligung von Pyridin in 1-3-Phosphoglycerinsäure übergehen (vgl. S. 109). Diese kann ihrerseits Phosphorsäure an das Adenylsäuresystem abgeben und selbst in 3-Phosphoglycerinsäure umgewandelt werden. Durch Phosphoglykomutasewirkung wird die 3-Phosphoglycerinsäure zu 2-Phosphoglycerinsäure umgelagert, welche in Phosphobrenztraubensäure weiterumgewandelt wird. Aus dieser kann Phosphorsäure unter Bildung von Brenztraubensäure abgespalten werden. Die frei gemachte Phosphorsäure kann von der intermediär gebildeten Glycerinaldehydphosphorsäure aufgenommen und zum Adenylsystem übergeführt werden, wodurch die Adenosintriphosphorsäure stetig regeneriert wird. Dadurch kommt ein ständiger Kreislauf der Phosphorsäure beim Kohlehydratabbau zustande.

Bei der Milchsäurebildung wird die Brenztraubensäure durch eine Reduktion in der oben angegebenen Weise in Milchsäure umgewandelt.

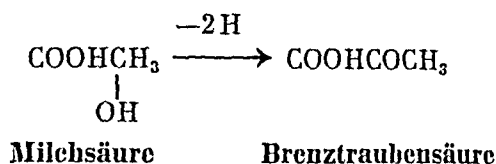
Bei der Dephosphorylierung der Phosphobrenztraubensäure werden grosse Energiemengen frei gemacht, welche den intermediären Stoffwechselprozessen zugute kommen.

Die Umwandlung der Hexosediphosphorsäure in Milchsäure wird aus dem nebenstehenden Schema ersichtlich.



## Die oxydative Abbauphase

Die in der Muskulatur beim anaeroben Abbau der Hexosephosphorsäureester gebildete Milchsäure kann ohne Mitwirkung von Sauerstoff nicht verschwinden. Während der Oxydation wird in erster Linie Brenztraubensäure durch Dehydrierung der Milchsäure wiedergebildet.

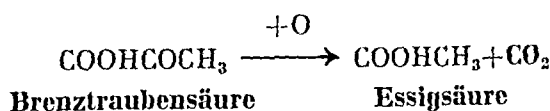


Die Brenztraubensäure ist ein wichtiges Intermediärprodukt beim Kohlehydratstoffwechsel. In der Norm ist sie in einer Menge von 0,5—1,5 mg% im Blute vorhanden, dessen Brenztraubensäuregehalt im Laufe der 24Stundenperiode normalerweise ziemlich konstant ist (vgl. S. 305).

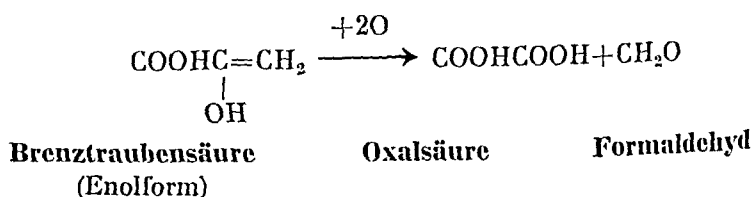
### Umwandlungsprozesse der Brenztraubensäure

Die oxydative Umwandlung der Brenztraubensäure kann auf mehreren Wegen erfolgen.

1. Durch oxydative Ketonspaltung werden Essigsäure und Kohlendioxyd gebildet.

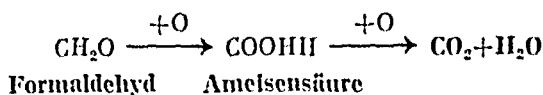


2. Aus der Enolform können durch stärkere Oxydation mit Lösung der Doppelbindung Oxalsäure und Formaldehyd entstehen.



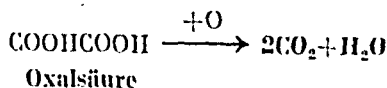
a) Eine *Azyloinkondensation* des Formaldehyds kann Glykolaldehyd (S. 92) entstehen lassen. Durch nachfolgende Aldolkondensation des Glykolaldehyds ist die Möglichkeit einer Hexose- und Polysaccharidbildung analog der Stärkeerzeugung bei den Pflanzen gegeben. Nach *Parnas* und *Baer* ist es möglich, dass die Glykogenbildung im Organismus auf diese Weise aus Oxydationsprodukten der Brenztraubensäure erfolgt (vgl. S. 129).

b) Der Formaldehyd kann durch weitere Oxydation auch in Ameisensäure übergehen. Diese wird leicht zu Kohlendioxyd und Wasser weiteroxydiert. Dabei wird Energie frei gemacht, welche evtl. bei einer Glykogenbildung ausgenützt werden kann.



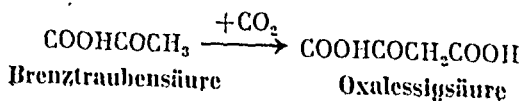
Ameisensäure wird normalerweise in zwischen 3,5 mg und 280 mg pro 24 Stunden wechselnden Mengen im Urin ausgeschieden (*Dakin, Strisower, Autenrieth*).

c) Die Oxalsäure, welche bei Oxydation der enoltransformierten Brenztraubensäure gebildet werden kann, wird wahrscheinlich teilweise zu Kohlendioxyd und Wasser weiteroxydiert (*Salkowski, Dakin und Hildebrandt*).



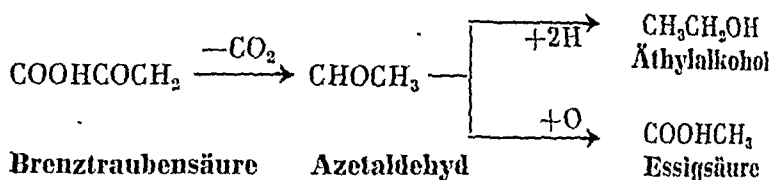
Nicht umgesetzte Oxalsäure wird im Urin ausgeschieden. Nach *Fürbringer* verlassen normalerweise etwa 20 mg Oxalsäure in 24 Stunden den Körper. Die ausgeschiedene Oxalsäure ist als Kalziumoxalat gebunden, welches von sauren Phosphaten des Harns in Lösung gehalten wird (*Hammarsten*).

3. In gewissen Fällen kann die Brenztraubensäure nach *Krebs* direkt Kohlendioxyd aufnehmen und in eine Dikarbonsäure, Oxalessigsäure, übergehen.

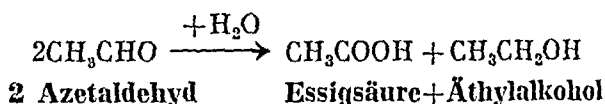


Zusammen können dann Brenztraubensäure und Oxalessigsäure Zitronensäure bilden, welche ein wichtiges Glied im Intermediärstoffwechsel ist (vgl. S. 133).

4. Durch *Dekarboxylierung* kann Brenztraubensäure in Azetaldehyd übergehen, welcher durch Oxydation in Essigsäure oder durch Reduktion in Alkohol umgewandelt werden kann.



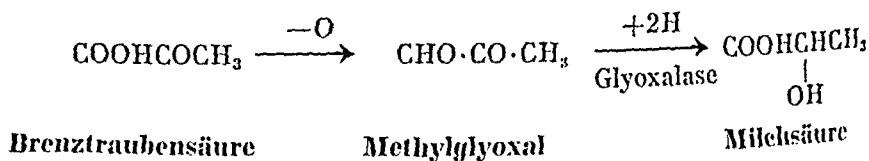
Azetaldehyd, der im Organismus gebildet wird, kann auch infolge der Wirkung einer in der Leber vorkommenden Aldehydmutase durch eine *Cannizaro-Umlagerung* in Essigsäure und Äthylalkohol übergeführt werden (*H. Fischer*).



Äthylalkohol findet sich normalerweise in kleinen Mengen — bis zu 50 mg% — im Blute und kommt auch im Urin vor. Unter pathologischen Verhältnissen kann Äthylalkohol infolge von Störungen im Zwischenstoffwechsel in erhöhter Menge auftreten (vgl. S. 318).

Ob die Brenztraubensäure im Organismus Essigsäure bildet, ist nicht mit Sicherheit bekannt. Sowohl Azetaldehyd wie Essigsäure kommen jedoch in kleinen Mengen normalerweise im Urin vor. *Stapp* und *Rothman-Manheim* geben die normale Menge des ausgeschiedenen Azetaldehyds mit 1,58—11,9 mg pro 24 Stunden an.

5. Durch Reduktion geht die Brenztraubensäure in Methylglyoxal über, welches durch die Wirkung von Glyoxalase in Milchsäure weiterumgewandelt werden kann.



Nach *Neuberg* und *Kobel* wird die Wirkung der Glyoxalase bei Anwesenheit von Zinkionen katalysiert.

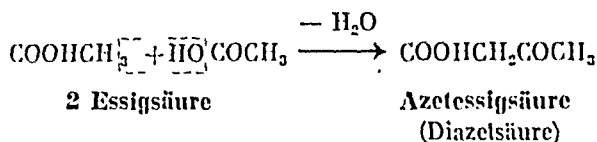
6. Weitere Umwandlungen der Brenztraubensäure siehe Zitronensäurezyklus (S. 133).

### Umsetzung der Essigsäure

Ein direkter oxydativer Abbau von Essigsäure im Organismus hat sich nicht nachweisen lassen. Bei Versuchen an Diabetikern mit peroralen Alkaliazetatgaben scheint eine Zunahme der Ketonkörperausscheidung stattzufinden.

Der oxydative Abbau der Essigsäure erfolgt vermutlich indirekt nach Zusammenkoppelung der Essigsäuremoleküle. Dabei bestehen drei Möglichkeiten:

a) Schliessen sich zwei Moleküle Essigsäure unter Wasseraustritt zusammen, so entsteht Azetessigsäure, welche bei gewissen Diabetesformen in grosser Menge auftritt.

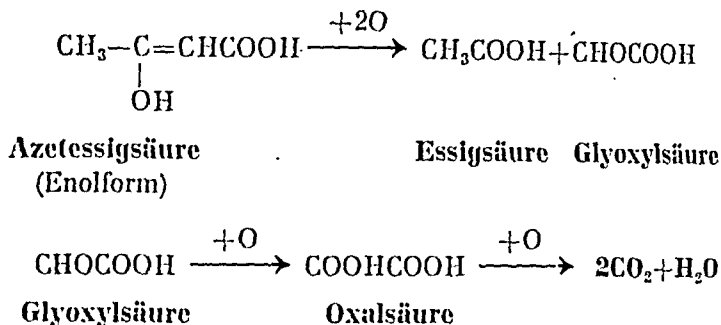


*Embden*, *Loeb*, *Friedmann* u. a. haben gefunden, dass Azetaldehyd und Essigsäure bei Perfusionsversuchen an glykogenarmen Lebern Azetessigsäure bilden;  $\beta$ -Oxybuttersäure konnte dagegen nicht nachgewiesen werden.

Da die Ketonkörperbildung auch mit dem Fettsäureumsatz zusammenhängt, kann das Ausgangsmaterial in den einzelnen Fällen verschieden sein. Der primäre Bildungsmechanismus für Azetessigsäure und  $\beta$ -Oxybuttersäure ist daher wahrscheinlich nicht derselbe, wenn auch die Ketonkörper dann sekundär ineinander umgewandelt werden können. Beim Diabetes liegt auch ein wechselndes Verhältnis zwischen den ausgeschiedenen Mengen von Azeton-Diazetsäure und  $\beta$ -Oxybuttersäure vor (vgl. S. 215).

Die weitere Umsetzung der Azetessigsäure erfolgt wahrscheinlich von der Enolform aus, mit Oxydation bei der Dop-

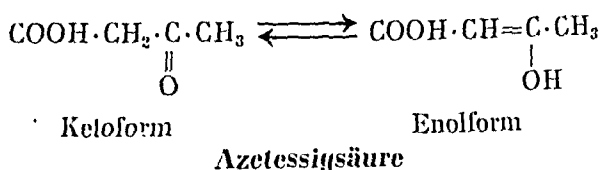
pelbindung. *Engfeldt* hat gezeigt, dass die Diazetsäure bei Oxydation mit Kaliumpermanganat in saurer Lösung Essigsäure und Glyoxylsäure entstehen lässt; die letztere wird zu Oxalsäure und dann zu Kohlendioxyd und Wasser weiteroxydiert.



Es ist möglich, dass der oxydative Abbau der Diazetsäure im Organismus auf dem Wege über Glyoxylsäure und Oxalsäure vor sich gehen kann.

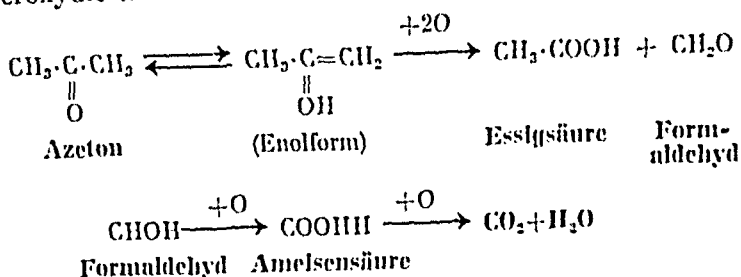
Eine endogene Oxalsäurebildung kann in diesem Falle die Alternative einer Ketonkörperbildung sein. Bleibt die Dekarboxylierung und Schlussoxydation der Oxalsäure aus, so wird die letztere im Körper angehäuft, sofern sie nicht in gesteigerter Menge im Harn ausgeschieden wird. Es ist von Interesse, dass *Loeper* bei einem Diabetiker Oxalsäure im Nervensystem nachgewiesen hat. *Brasher* u. a. haben auch beim Diabetes eine periodische Oxalsäureausscheidung abwechselnd mit Phosphaturie beobachtet.

Die Enolbildung der Diazetsäure geschieht durch Verschiebung des Wasserstoffs von Kohlenstoffatom 2 zu 3.

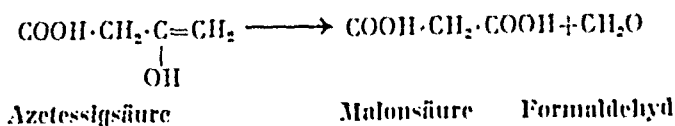


Es existiert noch eine Möglichkeit, und zwar die, dass die  $\text{CH}_3$ -Gruppe, wenn auch mit Schwierigkeit, ein Wasserstoffatom an eine CO-Gruppe abgibt. Dies ist z. B. bei starker Oxydation von Azeton der Fall, wobei Essigsäure und Formaldehyd

gebildet werden; letzterer wird zu Kohlendioxyd und Wasser weiteroxydiert.



Erfolgt die tautomere Wasserstoffverschiebung bei der Diazetsäure aus der  $\text{CH}_3$ -Gruppe, so kommt es zu einer Doppelbindung zwischen Kohlenstoffatom 3 und 4. Bei der weiteren oxydativen Spaltung können sich da aus der Diazetsäure Malonsäure und Formaldehyd bilden.



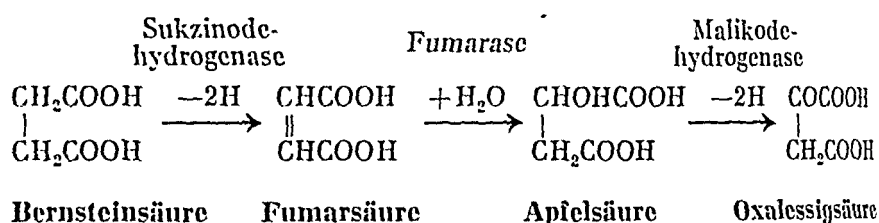
b) Vereinigen sich zwei Essigsäuremoleküle unter Dehydrierung, dann wird Bernsteinsäure gebildet. Nach *Thunberg* ist dies ein Weg beim weiteren oxydativen Abbau der Kohlehydrate. Bei gewissen Bakterien ist eine direkte Bernsteinsäurebildung aus Essigsäure nachgewiesen; bei höheren Lebewesen ist es dagegen nicht gelungen, eine Bernsteinsäurebildung aus Essigsäure mit Sicherheit zu beweisen. Nach *Cedrangola* und *Weil-Malherbe* ist dieselbe jedoch wahrscheinlich.

*Sven Forssman* (1941) hat gezeigt, dass auch im Tierorganismus Bernsteinsäure entsteht. Diese Bernsteinsäurebildung findet in der Leber und Muskulatur statt, die Bernsteinsäure tritt aber nicht in den Nieren auf. Die Bernsteinsäurebildung ist in der Leber am intensivsten.

Bei Perfusionsversuchen mit intravenöser Zufuhr von Bernsteinsäure an Katzen und Kaninchen hat *Forssman* gefunden, dass der grösste Teil der zugeführten Bernsteinsäure in den Nieren verbrannt wird. Nur eine kleinere Menge (höchstens

5 %) wird im Urin ausgeschieden. Bei Sauerstoffmangel oder Anwesenheit von Malonsäure, welche die Wirkung der Sukzinodehydrogenase hemmt, wird der Bernsteinsäureumsatz in den Nieren blockiert. Bei Anoxämie wird daher die an anderer Stelle im Körper gebildete Bernsteinsäure passiv durch die Nieren ausgeschieden.

Die Oxydation der Bernsteinsäure verläuft durch Dehydrierungsprozesse über eine Reihe C<sub>4</sub>-Säuren. Unter Mitwirkung dehydrierender Fermente, der Sukzinodehydrogenase, Fumarase und Malikodehydrase, entstehen sukzessive Fumarsäure, Apfelsäure und Oxalessigsäure.



Szent-Györgyi nimmt an, dass diese Serie von C<sub>4</sub>-Säuren ein bedeutungsvolles Wasserstofftransportsystem beim zellulären Atmungsprozess darstellt, indem dieselben Schritt für Schritt Wasserstoff abgeben. Ein ungestörtes Fortschreiten dieses Dehydrierungsprozesses ist demgemäss die Voraussetzung für den weiteren Oxydationsvorgang. Wird das Dehydrierungssystem gehemmt, so wird der Oxydationsprozess blockiert, und es kommt zu Störungen im normalen Kohlehydratumsatz.

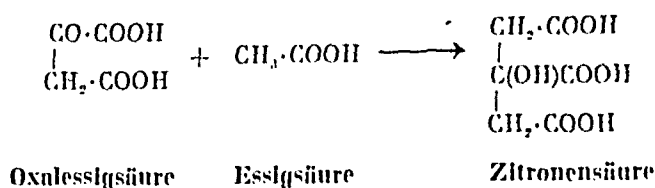
Eine derartige Blockierung findet bei Anwesenheit von Malonsäure statt, welche die Wirkung der Sukzinodehydrogenase bei der Umsetzung der Bernsteinsäure hemmt. Dieser hemmende Effekt der Malonsäure ist zuerst von Quastel (1926) nachgewiesen worden. Ein Überschuss an Malonsäure verhindert die oxydative Umwandlung der Bernsteinsäure. Eine ähnliche Wirkung haben auch  $\alpha$ -Ketoglutarsäure und Pyrophosphorsäure. Bei Anwesenheit dieser Säuren wird daher der Dehydrierungsprozess und damit die normale oxydative Umsetzung der Bernsteinsäure erschwert.

*Durch Bildung von dehydrasehemmenden Säuren im Verlauf des intermediären Stoffwechselprozesses kann gewisser-*

massen eine Selbstregulierung der biologischen Atmung durch Dämpfung des Umwandlungszyklus der  $C_4$ -Säuren eintreten.

Die Wirkung der Sukzinodehydrogenase scheint nach Hechter auch bei Anwesenheit von Zinkionen gehemmt zu werden.

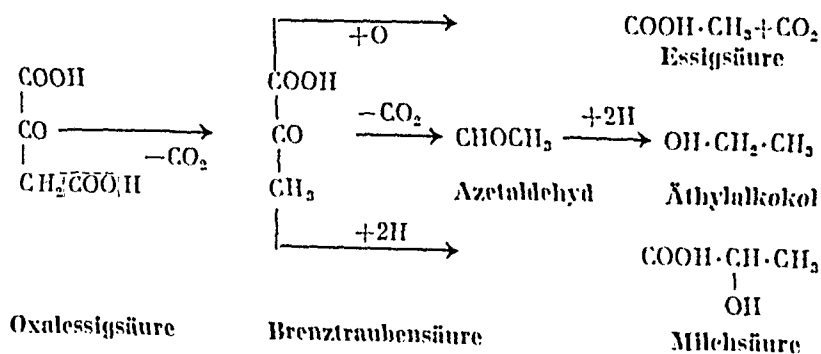
c) Essigsäure kann sich noch direkt mit Oxalessigsäure zu Zitronensäure verbinden.



### Umsetzung der Oxalessigsäure

Unter den Dehydrierungsprodukten der Bernsteinsäure nimmt die Oxalessigsäure eine Schlüsselstellung ein. Abgesehen von der Reaktionsbereitschaft der Oxalessigester gibt es mehrere Möglichkeiten für die weitere Umwandlung der Oxalessigsäure.

1. Durch einfache *Dekarboxylierung* aus der Ketoform geht die Oxalessigsäure in Brenztraubensäure über. Durch fortgesetzte Dekarboxylierung kann die so entstandene Brenztraubensäure Azetaldehyd bilden, oder durch Oxydation Essigsäure, evtl. durch Reduktion Milchsäure.

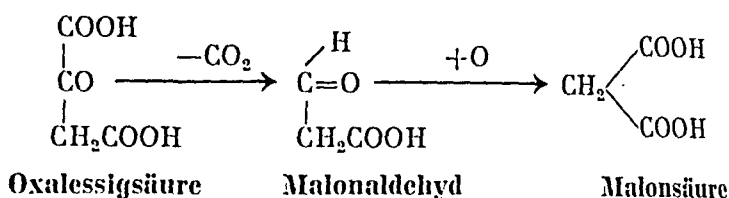


Essigsäure und Azetaldehyd können dann auf die oben angegebene Weise weiterumgewandelt werden.

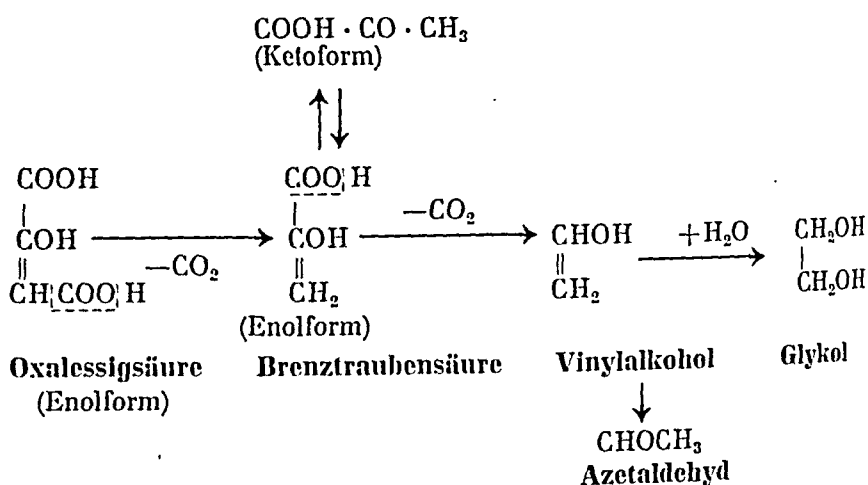
2. Ebenfalls durch Dekarboxylierung kann sich aus der



Oxalessigsäure auch Malonaldehyd bilden, der bei weiterer Oxydation Malonsäure liefert.



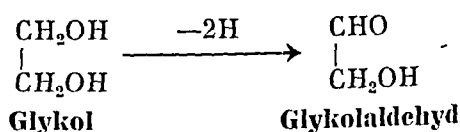
3. Eine hypothetische Umwandlung der Oxalessigsäure durch *Dekarboxylierung* aus der Enolform derselben ist zwar nicht nachgewiesen, besitzt aber Interesse, da hier ein Weg zur Glykogenbildung im Organismus gegeben ist. Bei dieser Umwandlung, die eine Enolasewirkung zur Voraussetzung haben muss, wird möglicherweise transitorisch Vinylalkohol gebildet, der durch Wasseranlagerung Glykol geben kann.



Der Vinylalkohol ist unbeständig, er wird leicht in Azetaldehyd umgelagert und kann daher nicht in freier Form auftreten.

### Glykogenbildung.

Bei Dehydrierung von Glykol entsteht Glykolaldehyd.

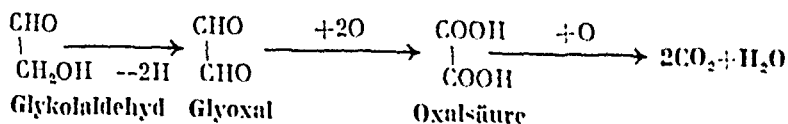


Durch Aldolkondensation und weitere Polymerisierung des Glykolaldehyds ist eine Möglichkeit zur Bildung von Hexosen und Polysacchariden gegeben. Eine auf diese Weise erfolgende Glykogenbildung ist denkbar, analog mit der Stärkeproduktion bei Pflanzen (vgl. S. 93 und 121).

Dieser synthetische Prozess setzt eine starke Dekarboxylierung und einen dehydrierenden Oxydationsprozess voraus. Es ist daher die Mitwirkung von Cokarboxylase — Vitamin-B<sub>1</sub>-pyrophosphat — erforderlich. Bei B<sub>1</sub>-Mangel treten auch Störungen im Kohlehydratstoffwechsel mit Anhäufung von Brenztraubensäure sowie herabgesetzter Glykogenbildung auf (s. S. 50). Bei unzulänglicher Oxydation kommt es ebenfalls zu verminderter Glykogenerzeugung mit gesteigertem Glykogenzerfall. Bei Asphyxie findet man auch den Glykogengehalt der Leber herabgesetzt.

Die Glykogenbildung aus Glykol und Glykolaldehyd ist durch direkte Perfusionsversuche nachgewiesen worden. *Baer, Embden, Grube, Parnas* u. a. haben gefunden, dass der Glykogengehalt der Leber nach Durchströmung mit Glykol, Glykolaldehyd, Glycerin, Glycerinaldehyd, Glycerinsäure und Milchsäure steigt.

Bleibt die Kondensation des Glykolaldehyds aus, so findet keine Glykogenbildung statt. Wenn der Glykolaldehyd dadurch Dehydrierung fernerhin oxydiert wird, dann entsteht Glyoxal. Dieses wird leicht zu Oxalsäure weiteroxydiert.

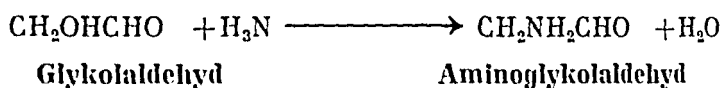


Die Oxalsäurebildung aus Glykolaldehyd kann gewissermaßen der Ausdruck einer misslungenen Glykogenbildung sein. Es ist möglich, dass die bei weiterer Oxydation der Oxalsäure frei werdende Energie zur Glykogensynthese ausgenutzt wird, und dass diese beiden Prozesse energetisch miteinander gekoppelt sind. Hierüber ist nichts bekannt.

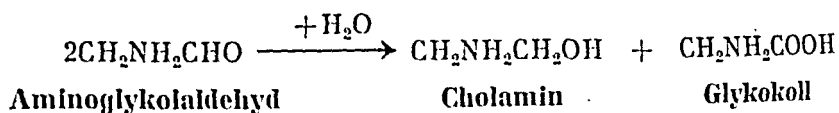
## Cholinbildung

Ein intermediäres Auftreten von Glykol und Glykolaldehyd bei der Umwandlung der Oxalessigsäure ist auch unter anderen Gesichtspunkten als dem der Glykogensynthese von Interesse. Die Bildung einer biologisch sehr wichtigen Substanz, des *Cholins*, ist hier denkbar.

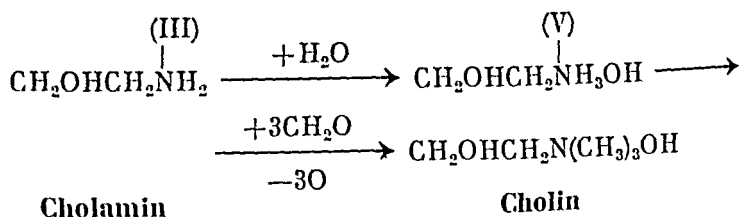
Durch Aminierung nach Aufnahme eines Moleküls Ammoniak geht der Glykolaldehyd in Aminoglykolaldehyd über.



Durch Wasseraufnahme macht der Aminoglykolaldehyd eine *Cannizzaro-Umlagerung* durch, wobei der entsprechende Aminoalkohol, *Cholamin*, und die Aminosäure, *Glykokoll*, gebildet werden.



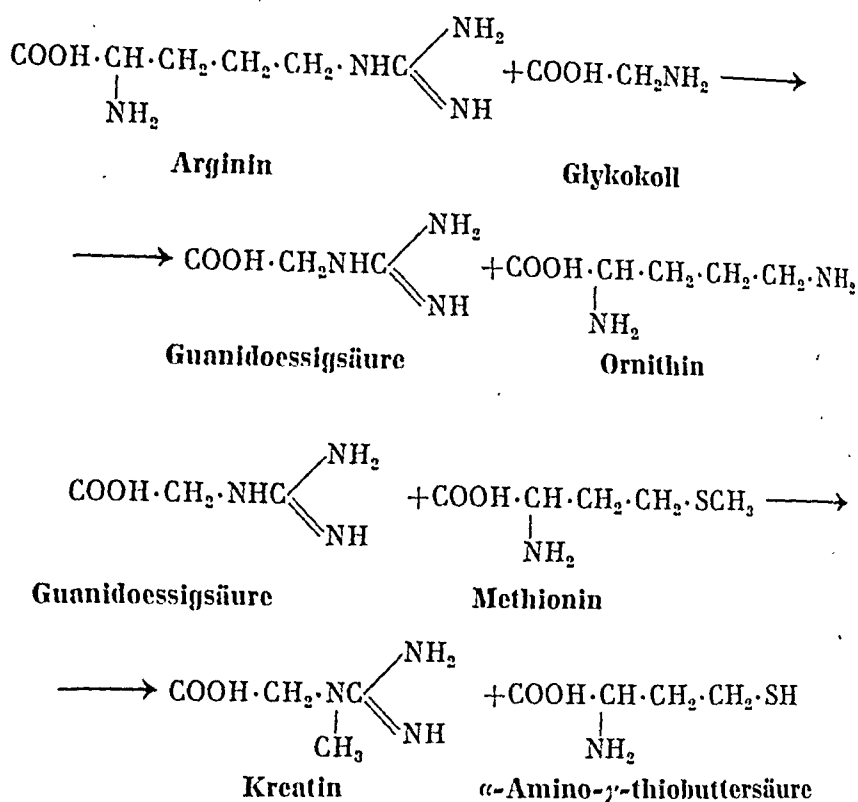
Das *Cholamin* kann nach Wasseranlagerung und Methylierung durch Aufnahme von drei Molekülen Formaldehyd unter Reduktion in *Cholin* übergehen.



Im Pflanzenreich hat *Trier* eine solche Cholinbildung aus Formaldehyd und Ammoniak nachgewiesen.

Beim Tierorganismus ist es dagegen unsicher, ob Cholin auf diese Weise gebildet werden kann. Zu einer normalen Entwicklung ist ein bestimmtes Cholinminimum in der Kost erforderlich.



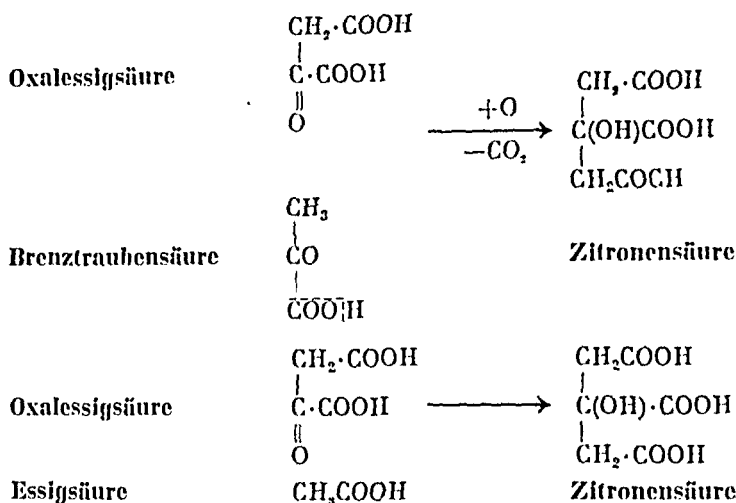


bei Ketonurie gesteigert ist. Nach *Brentano* soll die Kreatinurie vor der Azetonurie auftreten und ein Anzeichen für einen erhöhten Glykogenzerfall in der Muskulatur darstellen. Da aber auch Cholin bei der Ketonurie in vermehrter Menge ausgeschieden wird, ist es wahrscheinlicher, dass die gesteigerte Kreatinausscheidung mit einer Störung in denjenigen Prozessen zusammenhängt, welche zur Glykogenbildung führen.

Durch Beteiligung des Adenylsäuresystems kann das Kreatin Phosphorsäure unter Bildung von Kreatinphosphorsäure (Phosphagen) aufnehmen. Während des Ruhestadiums dient das Phosphagen als eine Phosphorsäurereserve in der Muskulatur, wird aber bei Muskelkontraktion dephosphoryliert. Die frei gemachte Phosphorsäure wird zur Phosphorylierung des Hexosemoleküls beim Kohlehydratabbau verwendet, und hierdurch wird das Kreatin zu einem vermittelnden Faktor beim Kohlehydratumsatz.

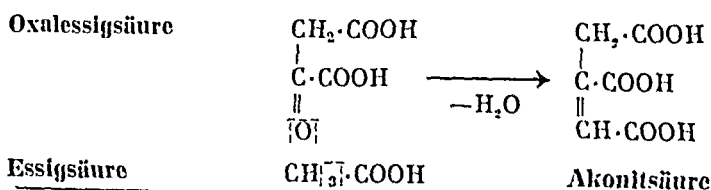
## Der Zitronensäurezyklus

Neben den oben behandelten drei Möglichkeiten für die Umwandlung der Oxalessigsäure gibt es noch eine vierte, die von *Martius* zuerst nachgewiesene Zitronensäurebildung durch Verbindung von Oxalessigsäure und Brenztraubensäure bei einer oxydativen Dekarboxylierung. Auch Essigsäure kann sich mit Oxalessigsäure zu Zitronensäure verbinden.



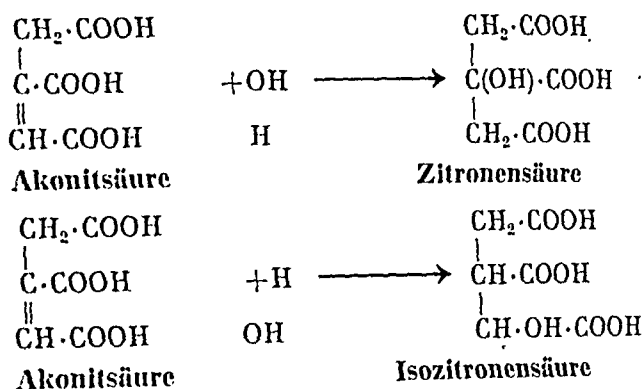
Nach *Martius* und *Knoop* wird die Zitronensäure über eine Serie von Zwischenprodukten, Akonitsäure, Isozitronensäure,  $\alpha$ -Ketoglutaräure, in Bernsteinsäure umgewandelt, aus welcher wieder Oxalessigsäure entsteht. Diese liefert dann durch Koppelung mit Brenztraubensäure von neuem Zitronensäure, worauf sich der Vorgang in einem ständigen Kreislauf wiederholt.

Es kann die Frage gestellt werden, ob sich nicht bei der Kondensation der Oxalessigsäure mit Brenztraubensäure und Essigsäure primär durch Koppelung an die Ketogruppe mit Wasseraustritt der Brenztraubensäure Akonitsäure bildet (*Ehrensward*<sup>1</sup>).

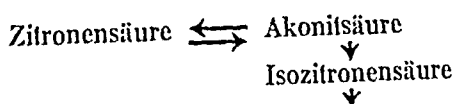


<sup>1</sup> mündliche Mitteilung.

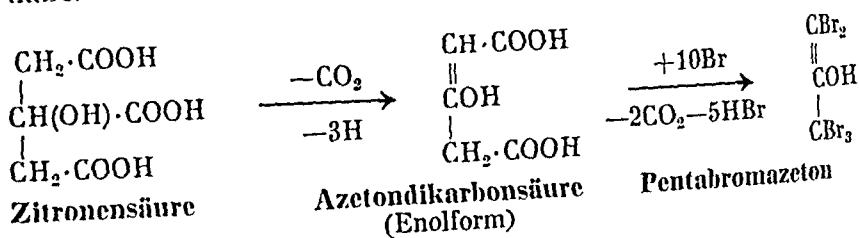
Aus der Akonitsäure kann dann durch Wasseranlagerung sowohl Zitronensäure als auch Isozitronensäure entstehen. Es



bildet sich dabei ein Gleichgewichtssystem zwischen Zitronensäure und Akonitsäure aus, welches mit der weiteren Umwandlung der Isozitronensäure verschoben wird:



Bei Oxydation in saurer Lösung findet eine Dekarboxylierung der Zitronensäure statt, welche bei Anwesenheit von Brom dabei in Pentabromazeton übergehen kann.



Durch diese Reaktion sind Möglichkeiten für eine quantitative Bestimmung der Zitronensäure gegeben (vgl. S. 440).

Bei der oxydativen Umwandlung im Zitronensäurezyklus wird Sauerstoff verbraucht, was man manometrisch messen kann. Hierdurch wird es möglich, Vorkommen und Menge der oxydierbaren Säuren quantitativ zu untersuchen (vgl. auch S. 442).

Bei der sukzessiven Umwandlung der C<sub>4</sub>-Säuren spielt sich eine Serie von Dehydrierungsprozessen ab. Nach Szen





*Györgyi* ist der Zitronensäurezyklus daher von Bedeutung für den Wasserstofftransport bei der biologischen Oxydation und ein Atmungssystem bei der Kohlehydratverbrennung, besonders in der Muskulatur. Nach *Krebs* ist der ungestörte Zitronensäureumsatz eine Voraussetzung für die normale Atmung der Muskulatur.

Der Verlauf des Zitronensäurezyklus wird aus der vorstehenden schematischen Darstellung ersichtlich.

## 2. Der Hexo-Pentosenabbau

Der oben beschriebene Hexosenabbau erfolgt durch Spaltung der Hexosemonophosphorsäure in zwei Triosen im Zusammenhang mit einer Oxydo-Reduktion. In wachsenden Geweben und vielleicht langsamer arbeitenden Organen ist der Kohlehydratabbau von anderem Typ, er verläuft dort nicht nur unter Triosenbildung, sondern auch durch eine Oxydation und Dekarboxylierung, mit Entstehung von Pentosen als erstem Glied und mit Äthylalkohol und vermutlich Glykol als Endprodukten. Der physiologisch-chemische Verlauf ist u. a. durch Arbeiten von *Dickens* (1938) bekannt.

Nach der Bildung von Hexose-6-monophosphorsäure ist der erste Schritt bei diesem Hexosenabbauprozess eine Oxydation wobei Phosphoglykonsäure entsteht. Hierzu ist die Mitwirkung eines *wasserstoffüberführenden Fermentsystems* erforderlich eines Triphosphopyridinnukleotids (*Warburgs Codehydrase II* mit seinem Fermentprotein, sowie eines *sauerstoffüberführenden Faktors*, welcher Laktoflavin sein kann (vgl. V. Abschnitt). Durch die Wirkung eines anderen Fermentproteins, des *Warburgschen Protein I*, in dem wasserstoffübertragenden System wird die Phosphoglykonsäure zu 2-Keto-phosphoglykonsäure oxydiert, welche dann dekarboxyliert wird, wobei sich eine Pentosemonophosphorsäure bildet. Diese ist nicht Arabinosphosphorsäure, wie es der Konfiguration nach zu erwarten wäre, sondern es findet eine Umlagerung mit Bildung von *d-Ribose-5-phosphat* statt. Diese Pentose ist in grossem Ausmass

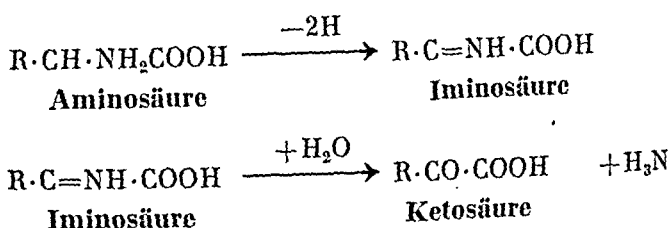


Die biologische Bedeutung dieses Abbauprozesses ist noch dunkel. Während der letzten Jahre hat man sich vielfach mit dieser Frage beschäftigt, nachdem *Olov Lindberg* (1941) in Eiern von Seetieren einen Phosphatester gefunden hat, der in diesen Zellen einen Hexosenzerfall entstehen lässt, welcher durch Hexo-Pentosenabbau erfolgt. Diesen Phosphatester hat *Lindberg* auch in der Leber und dem Gehirn von Säugetieren nachgewiesen. Der betreffende Ester ist stickstofffrei, enthält weder Hexose noch Pentosenreste, dagegen aber wahrscheinlich einen oxydierbaren Benzolring. Der *Lindberg*-Ester scheint als ein sauerstoffüberführender Faktor zu fungieren. Ausserdem dient derselbe möglicherweise auch bei dem eigentlichen Phosphorylierungsprozess als wirksames Agens. Von dem säurelöslichen Phosphat im Seeigeli sind ca. 14,5 % der Phosphorsäure in dem von *Lindberg* gefundenen Ester gebunden, etwa 31,5 % an Adenosin, und rund 54 % bestehen aus anorganischen Phosphaten. Der Hexo-Pentosenabbauprozess ist möglicherweise mit Lebensfunktionen des Zellkerns und mit dem Nukleinsäureumsatz verknüpft, aber hierüber ist noch nichts bekannt.

## Kohlehydrat- und Stickstoffumsatz

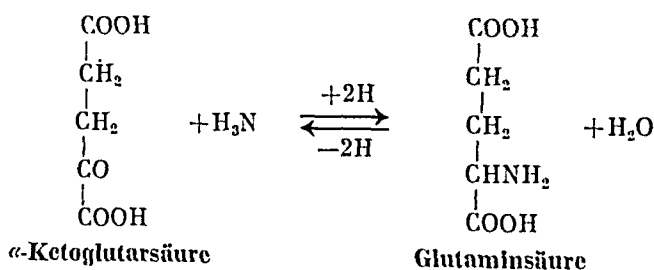
Bei der Desaminierung der Adenylsäure in der Muskulatur (S. 105) wird Ammoniak frei gemacht. Auch bei anderen Desaminierungsprozessen im Organismus entsteht Ammoniak.

Die unter dem Gesichtspunkt des Energiehaushalts wichtigste Ammoniakbildung ist die bei *oxydativer Desaminierung der Aminosäuren*. Bei dieser entstehen intermediär Iminosäuren, welche unter Bildung von Ketosäuren hydrolytisch desaminiert werden.

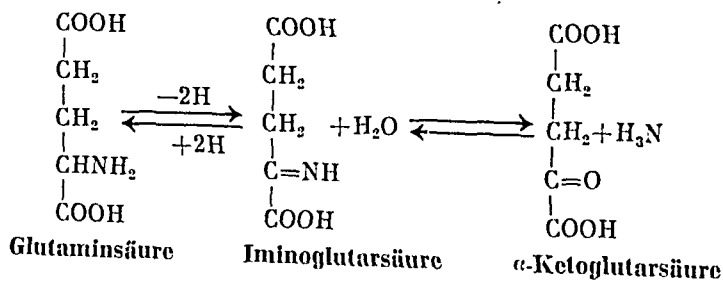


Diese Reaktion, welche von *Knoop* (1911) und *Emblen* (1912) zuerst nachgewiesen worden ist, hat *Krebs* eingehend studiert. Die oxydative Desaminierung von Aminosäuren findet bei höheren Tieren namentlich in der Leber, den Nieren und der Hirnrinde statt; in anderen Organen und bei niederen Lebewesen ist die Ammoniakbildung durch Aminosäurendesaminierung nur unerheblich. *Euler*, *Adler* und Mitarbeiter haben gezeigt, dass dehydrierende Atmungsfermente bei diesem Desaminierungsprozess wirksam sind.

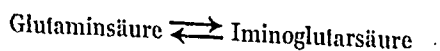
Bei der weiteren Umsetzung des frei gemachten Ammoniaks scheint die  $\alpha$ -Ketoglutar säure eine wichtige Rolle zu spielen.  $\alpha$ -Ketoglutar säure wird intermediär bei der Zitronensäureverbrennung gebildet. Sie wird durch Ammoniak anlagerung und Reduktion in Glutaminsäure umgewandelt. Der Vorgang ist reversibel.



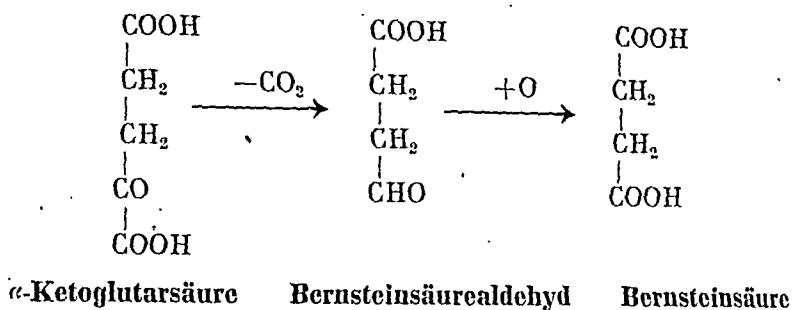
Bei Oxydation der Glutaminsäure entsteht wieder  $\alpha$ -Ketoglutar säure. Dabei wird intermediär Iminoglutar säure gebildet.



Nach *Krebs* und *Cohen* spielt das System

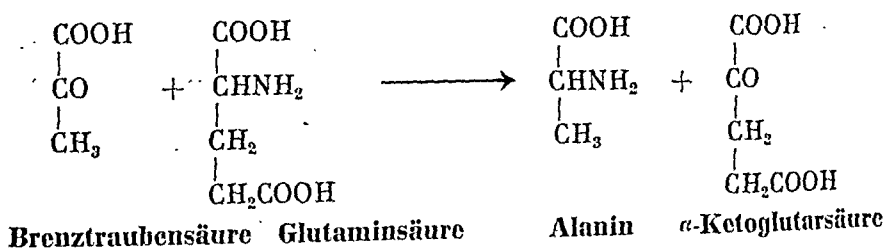


bei der weiteren Oxydation der  $\alpha$ -Ketoglutar säure die Rolle eines wasserstoffüberführenden Faktors. Dieser Prozess erfolgt durch eine oxydative Dekarboxylierung, wobei die  $\alpha$ -Ketoglutar säure in Bernsteinsäure übergeht.



Die Iminoglutar säurebildung aus der Glutaminsäure ist wahrscheinlich energetisch an den Übergang der  $\alpha$ -Ketoglutar säure in Bernsteinsäure gekoppelt. Dieser Prozess ist von Krebs und Cohen (1939) in den Nieren und der Herzmuskulatur nachgewiesen worden.

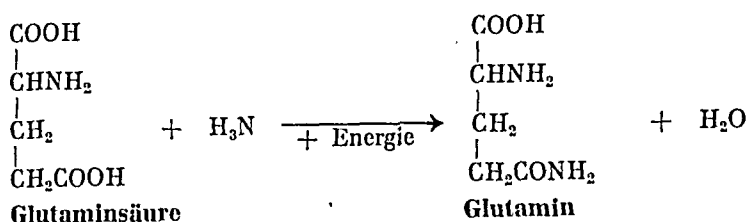
Braunstein und Kritzmann haben gezeigt, dass die Glutaminsäure bei Anwesenheit von Muskelextrakt ihr Ammoniak an  $\alpha$ -Ketosäuren abgeben kann, speziell an Brenztraubensäure und Oxalessigsäure. Hierbei werden die entsprechenden Aminosäuren, Alanin und Asparaginsäure, gebildet. Es entsteht da



wieder  $\alpha$ -Ketoglutar säure, welche neues Ammoniak aufnehmen und dasselbe auf andere Ketosäuren unter Bildung von Aminosäuren übertragen kann.

Die  $\alpha$ -Ketoglutar säure, welche beim oxydativen Abbau der Kohlehydrate intermediär gebildet wird, wird hierdurch auch zu einer für den Stickstoffumsatz im Organismus bedeutungsvollen Substanz.

Die Glutaminsäure kann im Gegensatz zu vielen anderen Aminosäuren weiterhin Ammoniak aufnehmen und in Glutamin übergehen.



Diese Glutaminsynthese ist von *Krebs* (1935) nachgewiesen worden und setzt eine intakte Zellstruktur sowie Energiezufuhr voraus. Die Glutaminsynthese erfordert die Anwesenheit von Sauerstoff und lässt sich unter anaeroben Verhältnissen nicht nachweisen. Namentlich in den Nieren erfolgt gelegentlich der Atmung des Nierengewebes nach *Krebs* eine intensive Glutaminbildung aus Glutaminsäure und Ammoniak.

Glutamin kann auch durch Abbau von Eiweisstoffen und Spaltung von Glutaminylpolypeptiden gebildet werden.

Unter Mitwirkung eines desamidierenden Enzyms, der *Glutaminase*, kann das Glutamin das aufgenommene Ammoniak wieder abgeben und sich in Glutaminsäure zurückverwandeln. Nach *Krebs* findet sich Glutaminase in allen Geweben und Organen, in welchen eine Glutaminsynthese stattfinden kann, im Gehirn, der Netzhaut, den Nieren, der Leber usw. Nach *Leut-hard* ist der Glutaminasegehalt in der grauen Hirnsubstanz und im Nierenparenchym besonders hoch. Die Fähigkeit der Nieren, Ammoniak zu bilden, hängt wahrscheinlich mit dem Glutaminasegehalt des Nierenparenchyms zusammen.

*Örström* (1939) hat eine starke Glutaminbildung aus Glutaminsäure und Ammoniak in dem befruchteten Seeigeelei gefunden, nicht aber in dem unbefruchteten Ei. Ob es der gesteigerte Atmungsprozess oder andere Faktoren im befruchteten Ei sind, welche die Ursache der lebhafteren Glutaminsynthese darstellen, ist nicht geklärt.

Die Ammoniakbildung in der lebenden Zelle wird daher wahrscheinlich in grossem Umfang von dem Verhältnis zwischen Glutaminsynthese und enzymatischem Glutaminzer-

fall geregelt. Bei herabgesetzter Atmung nimmt die Glutaminsynthese ab, wobei sich der enzymatische Glutaminzerfall steigert und die Ammoniakbildung zunimmt. Ebenso verhält es sich, wenn der Sauerstoff bei der zellulären Atmung zu einem anderen Zweck als zur Glutaminsynthese verbraucht wird, z. B. zur Oxydation von Ketonkörpern. Dieser Sachverhalt kann die vermehrte Ammoniakbildung in den Nieren gelegentlich der gesteigerten Ketonkörperverbrennung im Nierenparenchym beim ketonkörperbildenden Diabetes erklären (vgl. S. 248 u. 250).

Bei der Glutaminsynthese scheint nach *Örström* die Wasserstoffionenkonzentration von grosser Bedeutung zu sein: sie bleibt im Seeigelei bei einem pH-Wert unter 6 oder über 9 aus. Wenn die Glutaminsynthese bei sinkendem pH-Wert abnimmt, entsteht freies Ammoniak in gesteigerter Menge, welches bei der Ketosäureausscheidung als neutralisierender Faktor wirken kann. Da das Ammoniak ausserdem den Atmungsprozess im Nierenparenchym anregt, nehmen durch die Ammoniakbildung wahrscheinlich sowohl Ketonkörperverbrennung wie Resynthese des Glutamins zu.

Nach *Örström* ist der normale Glutamingehalt des Blutes  $9,6 \pm 0,30$  mg%. Beim Diabetes ist der Glutamingehalt des Blutes herabgesetzt. Die hier folgende Tabelle 9 nach *Örström* zeigt den Glutamingehalt des Blutes bei 8 Fällen von Diabetes (4 ohne Ketonurie vom Typus B, 4 mit ketonkörperbildendem Diabetes vom Typus A, vgl. VIII. Abschnitt).

TAB. 9.  
Glutamingehalt des Blutes bei Diabetes.

Diabetestypus	Glutamin mg %	Diabetestypus	Glutamin mg %
<i>Mit Ketonurie</i>		<i>Ohne Ketonurie</i>	
Martin O. ....	3,6	Karl L. ....	10,1
Frans O. ....	5,5	Oskar A. ....	5,5
Börje K. ....	3,7	Emilia L. ....	8,0
Helge H. ....	3,5	Emil N. ....	7,0
	Mittel 4,1 mg %		Mittel 7,7 mg %

Der Glutamingehalt des Blutes ist also beim Diabetes mit Ketonurie herabgesetzt. Wahrscheinlich hängt die Ammoniakbildung bei der Ketonurie mit einer ungenügenden Glutaminbildung oder einem gesteigerten Glutaminzerfall zusammen. Bei der Desaminierung des Glutamins wird wieder Glutaminsäure gebildet, welche ihrerseits durch oxydative Desaminierung in  $\alpha$ -Ketoglutarsäure übergeht. Diese wird gelegentlich des Ringprozesses des Zitronensäurezyklus leicht weiteroxydiert.

Auf diese Weise wird die Ketonkörperverbrennung von der Umsetzung des Glutamins abhängig, wobei das Ammoniak bei Ketonurie zu einem wirksamen Schutz für die Nieren wird. Die Ammoniak- $\beta$ -Oxybuttersäurebilanz stellt also einen wichtigen Faktor beim ketonkörperbildenden Diabetes dar, und der Quotient:

$$\frac{\text{Ammoniak}}{\beta\text{-Oxybuttersäure}}$$

im Urin bildet einen Massstab für die Fähigkeit der Nieren, die Ketonkörperbildung im Organismus zu bemeistern (vgl. VII. Abschnitt, S. 239).

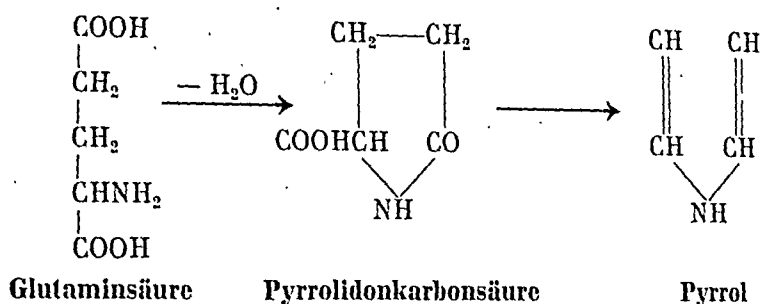
Die  $\alpha$ -Ketoglutarsäure ist ein beim Kohlehydratumsatz intermediär entstehender Ammoniakakzeptor. Sie kann das vorhandene Ammoniak unter Bildung von Glutaminsäure bzw. Glutamin binden. Diese Substanzen können ihrerseits Ammoniak an Ketokarbonsäuren unter Bildung von Aminosäuren abgeben, welche dann in neu gebildete Eiweisskörper eingehen können.

Wie unten näher besprochen werden wird, spielt nach Örstöm das Glutamin wahrscheinlich auch bei der Harnstoff-, womöglich noch bei der Harnsäurebildung eine Rolle. Wenn dies der Fall ist, dann kann eine gesteigerte Harnstoffbildung seitens des Systems  $\alpha$ -Ketoglutarsäure  $\rightarrow$  Glutamin durch Verschwinden der  $\alpha$ -Ketoglutarsäure aus dem oxydativen Umsetzungsprozess der  $C_4$ -Säuren eine Störung im Zitronensäureumsatz verursachen.



## Pyrrolbildung

Die Glutaminsäure kann durch Ringschluss zwischen der Aminogruppe und der  $\delta$ -Karboxylgruppe zu Pyrrolkernen führen.



Da Pyrrolkerne in den Häminen, Porphyrinen und Gallenfarbstoffen enthalten sind, ist die Glutaminsäure womöglich auch für die Bildung dieser Substanzen von Bedeutung. Über Pyrrolkörper in Atmungsfermenten vgl. S. 166.

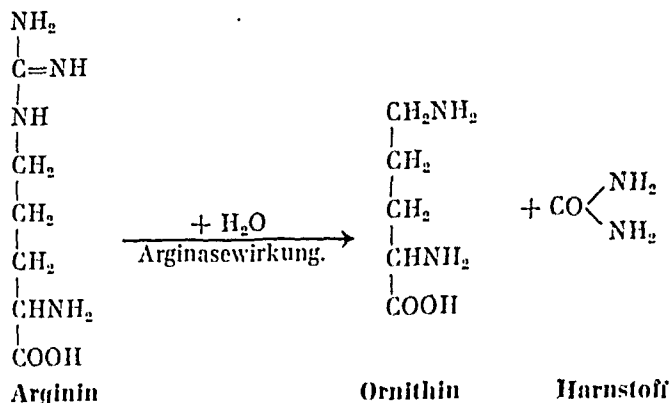
## Die Harnstoffbildung

Der überschüssige Stickstoff verlässt den Körper zum grossen Teil als Harnstoff. Die Verteilung des ausgeschiedenen Stickstoffs ist unter normalen Verhältnissen beim erwachsenen und neugeborenen Menschen nach *Sjöqvist* folgende:

	Erwachsene %	Neugeborene %
Harnstoff .....	84—91	73—76
Ammoniak .....	2—5	7,8—9,6
Harnsäure .....	1—3	3,0—8,5
übr. N-haltige Substanzen .....	7—12	7,3—14,7

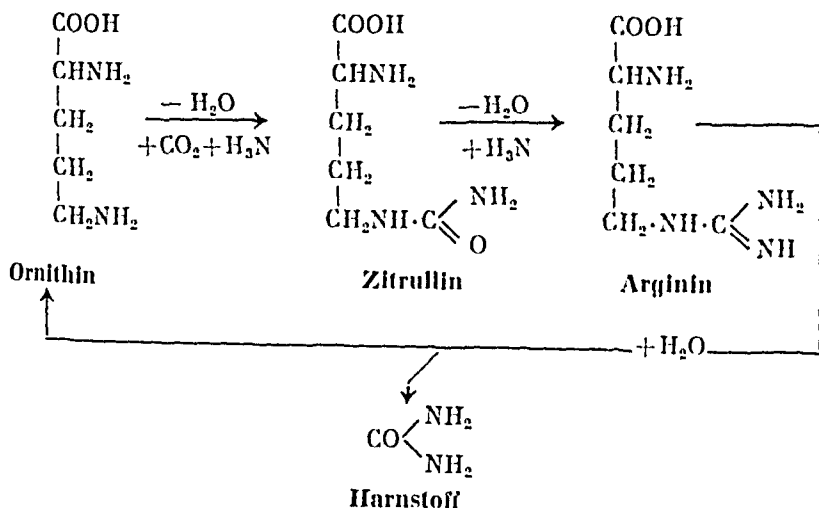
Die Harnstoffbildung findet hauptsächlich in der Leber statt. Durch Perfusionsversuche an überlebenden Lebern haben *Salaskin, Jansen* u. a. nachgewiesen, dass Glykokoll, Leuzin, Asparagin und andere Aminosäuren in der Leber in Harnstoff umgewandelt werden.

*Der Ornithinzyklus.* Das bei Desaminierungsprozessen frei werdende Ammoniak kann sich im Organismus nicht direkt



mit Kohlendioxyd zu Harnstoff verbinden. Diese Synthese muss durch Vermittlung katalysatorisch wirkender Substanzen erfolgen. Ein derartiger Körper ist das *Ornithin*. *Krebs* hat gezeigt, dass diese Aminosäure in der Leber Kohlendioxyd und Ammoniak aufnehmen und mit Zitrullin als Zwischenstufe *Arginin* bilden kann.

Durch die Wirkung der von *Kossel* und *Dakin* in der Leber und Niere gefundenen Arginase wird das Arginin in Ornithin und Harnstoff gespalten. Das wiedergebildete Ornithin kann von neuem Kohlendioxyd und Ammoniak aufnehmen, wobei wiederum Arginin entsteht, welches dann zerfällt. Den Verlauf veranschaulicht das hier folgende Schema.

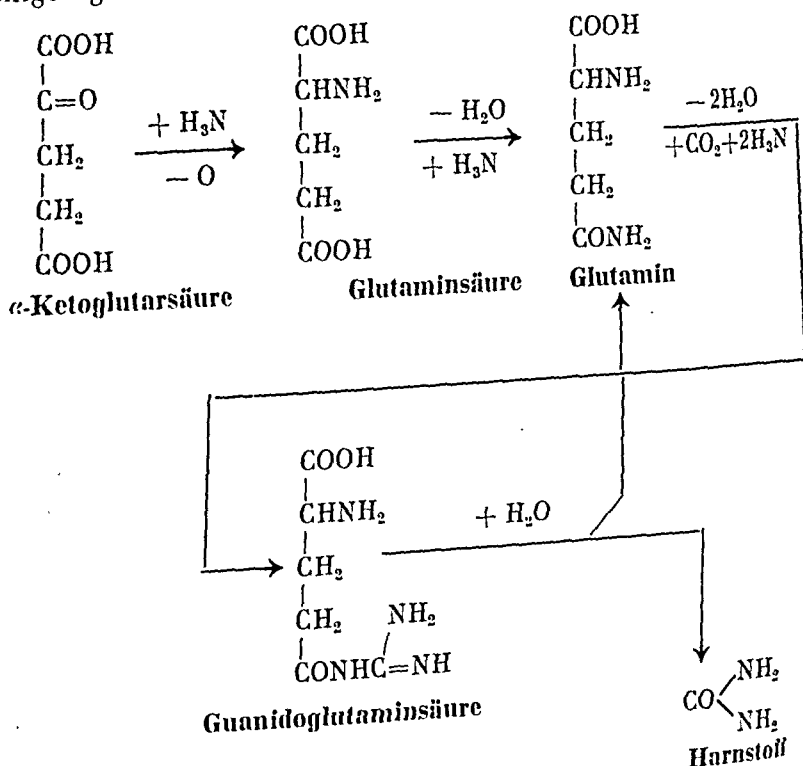


Bei Fütterungsversuchen mit Arginin fand *Thompson* die gesamte Stickstoffmenge des Arginins in dem ausgeschiedenen Harnstoff wieder. Der Überschuss an Ornithin muss daher im Organismus desaminiert und in Harnstoff umgewandelt werden.

**Der Glutaminzyklus.** Eine andere Form von Harnstoffbildung, welche nicht die Anwesenheit von Ornithin erfordert, ist von *Leuthard* und *Bach* angedeutet worden. Hier spielt wahrscheinlich das Glutamin eine für die Harnstoffbildung wesentliche Rolle.

Aus der  $\alpha$ -Ketoglutar säure kann durch Ammoniak anlage rung Glutamin entstehen. Nach *Örström* kann sich aus dem Glutamin durch Aufnahme von Kohlendioxyd und Ammoniak Guanidoglutaminsäure bilden. Durch hydrolytische Spaltung wird aus diesem Produkt Harnstoff abgespalten und Glutamin wiedergebildet.

Durch die  $\alpha$ -Ketoglutar säure kann also die Harnstoffbildung über das Glutamin mit der oxydativen Phase des Kohlehydrat abbaus verkoppelt werden. Der Verlauf geht aus dem hier eingefügten Schema hervor.



### Die Harnsäurebildung.

Es gibt im Organismus drei Typen von Harnsäurebildung.

1. Die exogene, wobei Harnsäure durch Spaltung von purinhaltigen Substanzen in der Nahrung entsteht.

2. Die endogene, wobei Harnsäure durch Zerfall der körpereigenen Nukleinsäuren gebildet wird.

3. Die Synthese von Harnsäure im Zusammenhang mit dem intermediären Stoffwechsel.

Die synthetische Harnsäurebildung, dürfte unter normalen Verhältnissen keine nennenswerte Rolle beim Menschen spielen, ist aber u. a. bei Vögeln und Reptilien der gewöhnlichste Weg der Stickstoffausscheidung. Bei pathologischen Zuständen beim Menschen, speziell bei Gicht, spielt dagegen nach *Örström* die synthetische Harnsäurebildung eine wichtige Rolle.<sup>1</sup> Diese Harnsäurebildung erfolgt nach *Örströms* Untersuchungen über Glutamin als Zwischenstufe. Bei Gicht ist der Glutamingehalt im Blut sehr erniedrigt, etwa 1/10 des normalen, und scheint im akuten Gichtanfall gleich Null sein zu können.

Der Mechanismus dieser Harnsäuresynthese verläuft nach *Örström* über eine Ureidbildung an  $\alpha$ -Stickstoffatomen im Gegensatz zu der Harnstoffbildung, wo die Ureidbildung bei  $\omega$ -Stickstoffatomen einsetzt. Durch Ringschluss kann das entsprechende Glutaminhydantoin entstehen. Die weiteren Zwischenstufen der Harnsäurebildung sind noch unbekannt.

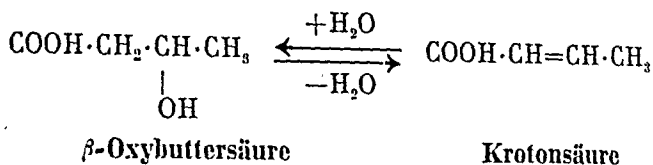
## Der Fettumsatz und seine Beziehung zum normalen Kohlehydratabbau

### Die Ketonkörper

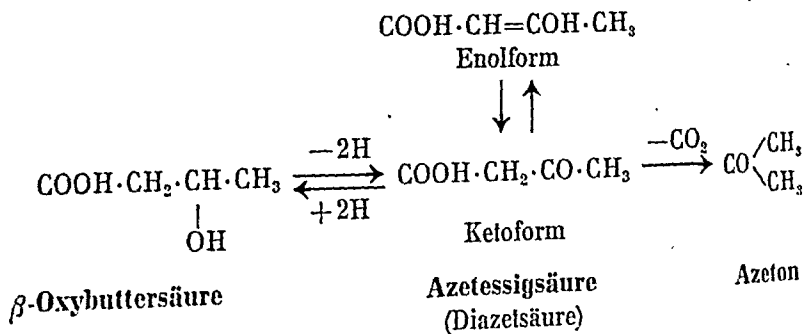
Beim Diabetes treten in vielen Fällen Ketonkörper, Azeton, Diazetsäure und  $\beta$ -Oxybuttersäure, im Urin auf. Das Erscheinen derselben ist mit Störungen im Fettumsatz eng verknüpft. Die Ketonkörper können sowohl bei Störungen in der Fettsynthese aus Kohlehydraten wie bei unvollständigem Abbau von Fettsäuren im Zusammenhang mit einer beeinträchtigten Glykogensynthese entstehen.

<sup>1</sup> Im Druck in Archiv für Chemie (Jan. 1913).

Die Ketonkörper können ineinander übergehen. Unter Wasseraustritt, z. B. bei Behandlung mit Schwefelsäure, wird die  $\beta$ -Oxybuttersäure in Krotensäure umgewandelt. Unter dem



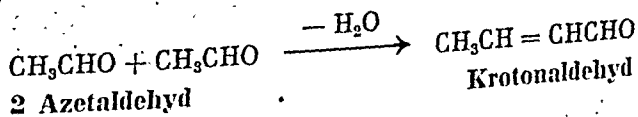
Einfluss starker Oxydationsmittel, beispielsweise der Chromsäure, wird aus der Krotensäure, ebenso wie aus der  $\beta$ -Oxybuttersäure, Diazetsäure. Umgekehrt wird  $\beta$ -Oxybuttersäure durch Reduktion von Diazetsäure gebildet. Nach *Neubauer* ist der Übergang der Diazetsäure in  $\beta$ -Oxybuttersäure ein reversibler Prozess, dessen Gleichgewichtszustand bei einer höheren  $\beta$ -Oxybuttersäurekonzentration liegt. Der Übergang der Azetonkörper ineinander wird von der folgenden Übersicht veranschaulicht:



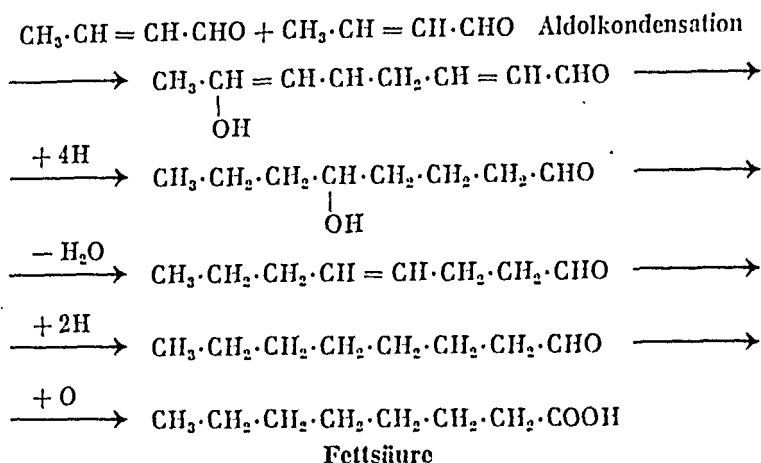
### Fettsäurebildung

Beim Kohlehydratabbau kann sich aus der Brenztraubensäure Azetaldehyd bilden.

Werden zwei Moleküle Azetaldehyd unter Wasseraustritt kondensiert, so entsteht Krotinaldehyd. Dieser Vorgang ist nach *Nencki* der erste Schritt bei der Bildung von Fettsäuren im Organismus.



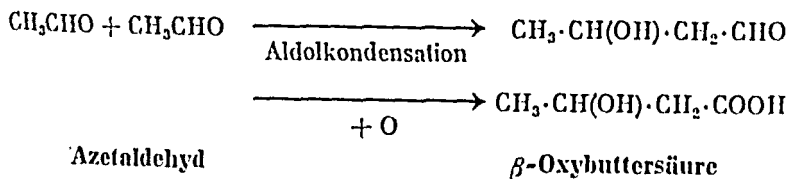
Durch eine Aldolkondensation können die langen Kohlenstoffketten der Fettsäuren aus dem Krotonaldehyd aufgebaut werden. Nach Nencki ist folgender Verlauf für die Fettsäuresynthese anzunehmen:



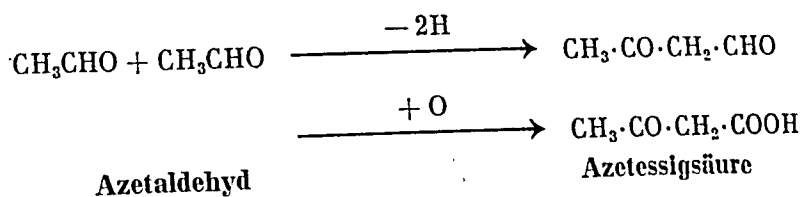
Fettsäuren, welche in Neutralfett und Lipide eingehen, lassen sich also direkt aus Umsetzungsprodukten der Kohlehydrate herleiten.

Die Zwischenstufen der endogenen Fettsäurebildung sind indessen nicht mit Sicherheit bekannt. Der Reichtum an Fettsäuren mit 18 Kohlenstoffatomen kann darauf hindeuten, dass die Fettsäurebildung möglicherweise durch Kondensation von drei Hexosemolekülen erfolgt, aber hierüber wissen wir nichts.

Bleibt die Krotonaldehydbildung aus, und findet an Stelle derselben die Aldolkondensation unmittelbar aus dem Azetaldehyd statt, so entsteht  $\beta$ -Oxybuttersäurealdehyd. Dieser kann zu  $\beta$ -Oxybuttersäure weiteroxydiert werden.



Wenn im Zusammenhang mit der Kondensation eine Dehydrierung eintritt, wird Azelessigsäure gebildet.



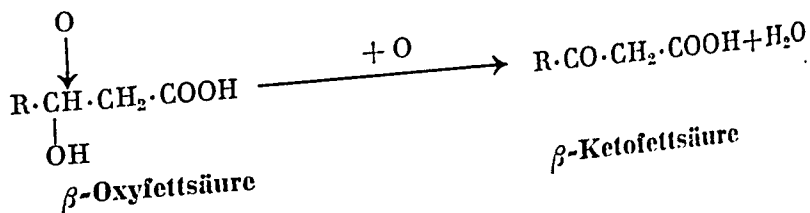
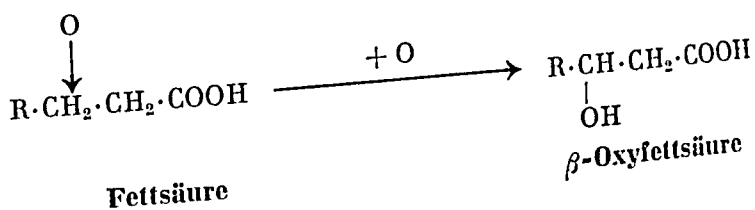
Die Ketonkörperbildung kann in diesem Falle als eine unvollständige Fettsäuresynthese betrachtet werden. Die Störung des normalen Vorgangs besteht dabei in dem Ausbleiben der Anfangskondensation zweier Azetaldehydmoleküle unter Wasseraustritt, wodurch die Bildung des ungesättigten Krotionaldehyds nicht erfolgen kann.

## Fettsäureabbau

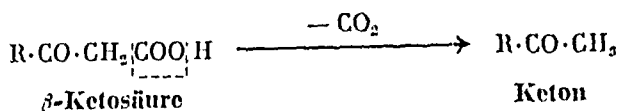
### Die $\beta$ -Oxydation

Beim Hunger, wo verfügbare Kohlehydrate fehlen, muss Glykogen aus Fett gebildet werden. Die Glyzerinkomponente des Fetts kann dabei ohne weiteres in Glykogen umgewandelt werden.

Die langen Kohlenstoffketten der hochmolekularen Fettsäuren müssen dagegen beim Fettumsatz in niedermolekulare Bruchstücke zerlegt werden. Durch Fütterungsversuche mit phenylsubstituierten Fettsäuren hat *Knoop* nachgewiesen, dass der Abbau der Fettsäuren durch eine Oxydation am  $\beta$ -Kohlenstoffatom vor sich geht. Dabei bildet sich in erster Linie eine  $\beta$ -Oxyfettsäure, welche bei fortgesetzter Oxydation eine  $\beta$ -Ketofettsäure entstehen lässt.



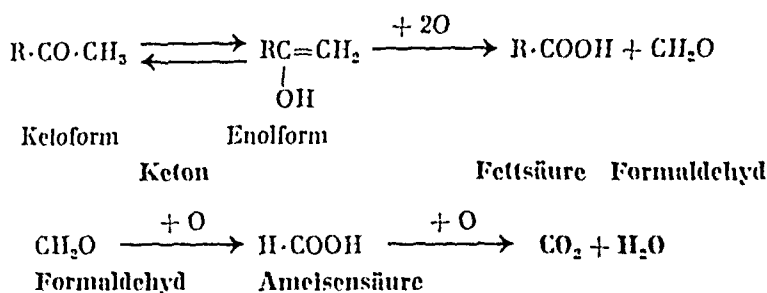
Aus  $\beta$ -Ketofettsäuren wird leicht Kohlendioxyd aus der Carboxylgruppe abgespalten, wobei ein Keton entsteht.



Bei weiterer Oxydation verschwindet die  $\text{CH}_3$ -Gruppe, und es wird wieder eine Fettsäure gebildet, in welcher eine  $\beta$ -Oxydation von neuem stattfinden kann.

Die Oxydation der  $\text{CH}_2$ -Gruppe wird wahrscheinlich von einer Wasserstoffverschiebung durch Enolasewirkung eingeleitet, mit Entstehung einer Doppelbindung, welche für die Oxydation leichter zugänglich ist.

Man kann folgenden Verlauf annehmen:



Bei Oxydation von Ketonen wird also Ameisensäure abgespalten, welche leicht zu Kohlendioxyd und Wasser oxydiert wird.

Blixenkrone-Møller hat u. a. den Sauerstoffverbrauch und den respiratorischen Quotienten bei Perfusionsversuchen an Lebern pankreasloser Katzen untersucht. Er kam zu dem Schluss, dass die langen Fettsäurekohlenstoffketten beim Abbau zunächst in mehrere kurze Ketten mit je 4 Kohlenstoffatomen zerfallen, welche der Buttersäure entsprechen. Erst in diesem Stadium beginnt die  $\beta$ -Oxydation. Bei mangelhafter Oxydation entsteht  $\beta$ -Oxybuttersäure, Diazetsäure oder Azeton, je nachdem, wie weit der Oxydationsprozess fortschreitet. Aus jedem Molekül einer höheren Fettsäure werden hierdurch mehrere Moleküle Azetonkörper gebildet.



Ist der Fettsäureabbau bis zur Bildung von Azetessigsäure fortgeschritten, dann kan aus dieser nach Enoltransformierung entweder Glyoxylsäure, oder nach Dekarboxylierung Formaldehyd abgespallet werden (vgl. S. 124). In beiden Fällen bleibt Essigsäure übrig. Diese scheint im Organismus nicht direkt verbrannt werden zu können (s. S. 123).

Die Verbrennung der Ketonkörper geht nach *Snapper* und *Grünbaum* vor allem in den Nieren, aber auch in der Muskulatur vonstatten. Bei Perfusionsversuchen an Hundemuskulatur haben *Snapper* und *Grünbaum* gefunden, dass eine Verbrennung von Azelessigsäure und  $\beta$ -Oxybuttersäure stattfinden kann. Der Verlauf im einzelnen ist nicht geklärt.

*Toeniessen* und *Brinkmann* haben nachgewiesen, dass Brenztraubensäure in der Muskulatur des Kaninchens in Bernsteinsäure umgewandelt wird. Essigsäure und Azetaldehyd bewirken nicht diese Bernsteinsäurebildung. Wird also bei der Verbrennung der Azelessigsäure Essigsäure gebildet, so kann diese wahrscheinlich nicht in der Muskulatur direkt in Bernsteinsäure verwandelt werden. Die weitere Umwandlung der Essigsäure erfolgt möglicherweise indirekt über den Zitronensäurezyklus in Verbindung mit dem oxydativen Abbau der Kohlehydrate (vgl. S. 135). Dadurch kann womöglich die antiketogene Wirkung der Kohlehydrate erklärt werden.

*Blixenkrone-Møller* hat ferner gefunden, dass die Ketonkörperbildung in den einzelnen Lebern verschieden stark ist. Sie wechselt mit dem Glykogengehalt. In glykogenreichen Lebern ist die Ketonkörperbildung geringer, sie nimmt aber zu, wenn die Leber arm an Glykogen ist, namentlich wenn sie dabei fettreich ist.

Eine herabgesetzte Glykogenbildung in der Leber kann der Ausdruck ungenügender Oxydationsprozesse sein. Diese üben da auch eine Rückwirkung auf den Fettumsatz aus, sowohl beim Abbau der Fettsäuren, als auch beim Aufbau derselben aus intermediären Stoffwechselprodukten. Der Ketonkörperbildung kommt dabei eine verschiedene biologische Bedeutung zu, je nachdem, ob dieselbe durch einen unvollständigen Fettsäureabbau oder durch eine unzulängliche Fettsäuresynthese zustande gekommen ist. Die fetten und die mageren ketonkör-

perbildenden Diabetikertypen sind möglicherweise eine klinische Manifestation des verschiedenen primären Bildungsmodus der Ketonkörper.

Der Zusammenhang zwischen dem Glykogengehalt der Leber und der Azetonkörperbildung ist unklar. Es ist nicht lediglich die Menge des Glykogens in der Leber, welche für die Ketonkörperbildung bestimmend ist.

*Vendég* hat bei Hungerversuchen an Hunden am 3. Hungertage einen geringeren Glykogengehalt als 1 % in der Leber ohne nennenswerte Ketonurie gefunden. Nach 3wöchiger einseitiger Fettkost tritt dagegen eine starke Ketonkörperbildung ein. Die Leber kann da über 2 % Glykogen enthalten, ist aber gleichzeitig fettreicher. *Vendég* konstatierte bei seinen Versuchen, dass das normale Tier bei fettreicher, kohlehydratarmer Nahrung während der ersten 2—3 Wochen eine intensive Zuckerbildung aus Fett aufwies. Normalerweise tritt da keine stärkere Ketonurie auf. Erst nach 3 bis 4 Wochen macht sich eine stärkere Ketonkörperbildung bemerkbar. Da steigt auch der Fettgehalt der Leber. Die Fähigkeit des Leberfetts, Zucker zu bilden, kan nach *Vendég* bis auf ein Sechstel der Norm herabgesetzt sein. Die Ketonkörperbildung wäre da als ein Ausdruck für die Unfähigkeit der Leber, Fett in Kohlehydrate umzuwandeln, aufzufassen.

Das Insulin wirkt nach *Vendég* während der ersten und zweiten Phase der Fettkost verschieden. In der ersten Periode, in der die Leberfunktion verhältnismässig ungeschädigt ist, würde das Insulin die Menge des Leberglykogens vermindern, die des Muskelglykogens aber zum Steigen bringen. Während der zweiten Periode dagegen, wo die Zuckerbildungsfunktion der Leber durch die anhaltende Fettkost geschädigt ist, wird weder die Menge des Leberglykogens noch die des Muskelglykogens beeinflusst, obgleich das Leberfett unter der Einwirkung von Insulin abnimmt. Blutzuckergehalt und Zuckerausscheidung lassen jedoch bei der Insulinwirkung eine Tendenz zum Steigen erkennen, was wahrscheinlich auf eine Zuckerbildung aus dem verschwindenden Leberfett zurückzuführen ist.

Die verschiedene Wirkung von Insulin während der ersten und zweiten Phase der Fettfütterung deutet darauf hin, dass

*Zuckerbildung und Glykogenspeicherung zwei teilweise getrennte Prozesse sind.* Die Glykogensynthese ist der empfindlichere Vorgang, welcher zuerst eine dauernde Schädigung erfährt. Dieselbe würde von Insulin nicht beeinflusst werden.

*Vendégs* Untersuchungen sind dadurch zum Teil unvollständig geblieben, dass die durch die rhythmische Leberfunktion verursachten normalen Variationen keine Berücksichtigung gefunden haben. Werden die Schlussfolgerungen *Vendégs* bestätigt, so sind diese Untersuchungen von grossem Interesse. Sie geben direkt über den schädlichen Einfluss einer anhaltenden Kohlehydratentbehrung auf die Glykogenbildungsfunktion der Leber Aufschluss.

Wenn mehr Ketonkörper im Organismus gebildet werden, als verbrannt werden können, wird der Überschuss im Urin oder, nach Umwandlung in Azeton, teilweise mit der Respirationsluft ausgeschieden. Kann nicht alles beseitigt werden, so kommt eine Anhäufung von Ketonkörpern im Blut und in den Geweben zustande. Die dabei auftretende Azidose wird im VII. Abschnitt näher besprochen werden.

Die Ketonkörperausscheidung hängt nicht nur von der Ketonkörperbildung ab, sondern auch von der Ketonkörperverbrennung im Organismus. Die periodische Ketonkörperbildung im Zusammenhang mit der rhythmischen Lebertätigkeit braucht sich daher nicht stets in der Ketonurie widerzuspiegeln, denn eine zeitweise gesteigerte Ketonkörperverbrennung kann hierbei die periodische Bildung verschleiern. Es ist da selbstverständlich nicht möglich, durch die Ketonurie zu entscheiden, ob die Ketonkörperbildung periodisch erfolgte oder nicht (vgl. S. 37).

Das Vorkommen von Ketonkörpern im Harn ist beim Diabetes ein ungünstigeres Zeichen als Zuckerdiurese und Blutzuckersteigerung. Es liegt wahrscheinlich eine Störung in denjenigen Reaktionsvorgängen vor, welche zur Zuckerbildung aus Fett führen. Ausserdem ist die Ketonkörperbildung ein Hinweis auf eine verschlechterte Glykogenenerzeugung in der Leber. Nach *Vendég* erleichtert das Insulin die Umwandlung des Fells in Zucker, es scheint aber dagegen die Glykogenbildungsfähig-

keit des Organismus nicht *direkt* zu steigern. Diese ist eine von der Zuckerbildung getrennte Funktion.

Beim Diabetes findet man auch im Einklang hiermit, dass das Insulin in vielen Fällen die Ketonkörperausscheidung rasch herabdrückt, welche aufhört, während die Zuckerausscheidung zunächst unverändert bleibt oder sogar steigt (vgl. Abb. 99 S. 379).

Es ist eine beim Beginn der Insulinbehandlung häufige Beobachtung, dass der Insulinbedarf nach dem Verschwinden der Azidose Schritt für Schritt geringer wird. Dies zeigt sich u. a. in fortschreitender Verminderung der Zuckerausscheidung bei konstanter Insulindosis und Nahrungszufuhr. Das Phänomen kann der Ausdruck einer durch die Insulinbehandlung allmählich verbesserten Glykogenbildung sein (s. Abb. 100 S. 381).

Wird die Kohlehydratzufuhr bei einem ketonkörperbildenden, mit Insulin behandelten Diabetiker konstant beibehalten, die Insulindosis aber sukzessive verkleinert, so bleibt die Zuckerausscheidung relativ unverändert und entspricht der zugeführten Kohlehydratmenge. Die Ketonkörperausscheidung weist dagegen eine starke Zunahme auf, wenn die Insulindosis herabgesetzt wird (s. Abb. 56 S. 275). Auch dieser Sachverhalt kann darauf hindeuten, dass es der Übergang des Fetts in Zucker ist, welcher bei mangelnder Insulinwirkung in erster Linie erschwert wird. Dies ist jedoch sicherlich nur eine Partialfunktion des Insulins im Organismus (vgl. S. 316).

### *Die $\omega$ -Oxydation*

Die Oxydation der Fettsäuren erfolgt nicht immer durch eine  $\beta$ -Oxydation mit Ketosäurebildung. In gewissen Fällen findet eine Bildung von Dikarbonsäuren durch Oxydation der Endmethylgruppe der Fettsäuren statt. Diese  $\omega$ -Oxydation hat Verkade nach Fütterungsversuchen mit 10 und 11 Kohlenstoffatome enthaltenden Fettsäuren nachgewiesen. Dabei waren die entsprechenden Dikarbonsäuren im Urin zu finden.

Mazza hat gezeigt, dass Fettsäuren bei Anwesenheit von Leber- und Nierensubstanz zu Dikarbonsäuren oxydiert werden können, welche in Diketo-Dikarbonsäuren weiterumgewandelt

werden. Es ist unsicher, ob diese  $\omega$ -Oxydation in grösserem Umfang im Organismus stattfindet, und unbekannt, ob sie für die intermediären Stoffwechselprozesse Bedeutung besitzt.

### *Biochemische Transformationsprozesse*

Einige der Transformationsprozesse des Zwischenstoffwechsels sind in dem folgenden Schema zusammengestellt. Es sei ausdrücklich betont, dass dasselbe nichts anderes darstellen soll, als ein Arbeitsschema zum weiteren Studium der diabetischen Stoffwechselstörung. Es ist daher keineswegs als eine Wiedergabe endgültig gesicherter Reaktionsverläufe zu betrachten. Das Fortschreiten der Reaktionen ist sicherlich nicht unbedingt an irgendwelche bestimmten Bahnen gebunden, sondern es gibt wahrscheinlich mehrere Reaktionswege, welche je nach den verschiedenen Bedürfnissen des Organismus wechseln. Der Lebensprozess ist ein *energetisches* Problem. Die konstitutionschemische Stofftransformation ist lediglich der Ausdruck eines Energieaustausches zwischen verschiedenen Substanzen. Abhängig von den Funktionen des Körpers wird bei dieser Stoffumformung Energie frei gemacht oder gespeichert. Alle die abnormen Ausscheidungsprodukte, welche man im Harn eines Diabetikers finden kann, sind sicher nicht Zeichen einer lokalisierten primären Störung in einem bestimmten Reaktionssystem, sondern eher als Indikatoren für einen veränderten Energieaustausch in den gekoppelten Systemen durch Störungen der oxydativen Prozesse aufzufassen. Das Diabetesproblem wird hierdurch zu einem energetischen Problem von äusserst komplizierter Art, mit Prozessen, die in verschiedenen Organen gestört sind.





## V. ABSCHNITT

# Der Asphyxiediabetes und die biologischen Oxydationsprozesse.

Glykogenbildung und Kohlehydratverbrennung im Organismus erfolgen, wie wir in vorangehenden Abschnitten kennengelernt haben, über eine Serie von intermediären Zwischenstufen. Durch gekoppelte Oxydo-Reduktions- und andere Umwandlungsprozesse, welche das Vorhandensein biologisch wirksamer Fermentsysteme erfordern, geht die normale Kohlehydratverbrennung ihren Gang, bis endlich Kohlendioxyd und Wasser entstehen. Will man sich vom Mechanismus des Kohlehydratabbaus ein klareres Bild machen, so muss man sich zunächst über die Wirkung der biologischen Oxydationsfermente unterrichten.

## Der Asphyxiediabetes

Da die synthetischen Prozesse an den oxydativen Abbau der Kohlehydrate gekoppelt sind, kommt es bei Störungen im Kohlehydratabbau auch zu solchen der Glykogenbildung. Dabei kann eine Anhäufung von Glykose und unvollständig umgewandelten Abbauprodukten stattfinden, wodurch ein Diabetes mit Ausscheidung von Zucker sowie auch von nicht in normaler Weise verarbeiteten Stoffwechselprodukten zustande kommt.

Araki (1891) fand, dass bei Sauerstoffmangel und Erstickung ein Diabetes mit abnormer Glykosebildung im Organismus auftrat. Er konnte dabei auch eine regelwidrige Milchsäurebildung nachweisen. Dieser *Asphyxiediabetes* lässt bei gesteigerter Sauerstoffzufuhr nach oder verschwindet.

Bei der Erstickung entstehen ebenfalls toxische Produkte, welche diabetesauslösend wirken. Yamakami hat beobachtet,



dass nach Einspritzung von Blut erstickender Tiere bei normalen Tieren eine Blutzuckersteigerung eintritt.

Bei *Kohlenoxydvergiftung* mit Blockierung des sauerstoffübertragenden Hämoglobins tritt nicht selten eine Glykosurie auf, welche oft einige Zeit anhält, auch nachdem sonstige akute Vergiftungssymptome abgeklungen sind (*Ewald*).

Ein diabetischer Zustand entwickelt sich bei hochgradigem Sauerstoffmangel mit Asphyxie in Geweben und Organen. Bei anhaltendem Sauerstoffmangel tritt im Anfangsstadium ein Sinken des Blutzuckerspiegels ein, welches schon *Claude Bernard* beobachtet hatte.

Die Angaben über Veränderungen des Blutzuckerspiegels in grösseren Höhen mit niedrigem Luftdruck widersprechen einander. *Aggazotti* u. a. haben Senkungen des normalen Blutzuckergehalts gefunden; *Araki*, *Kellaway*, *Eadie* und *Macleod* u. a. dagegen einen Anstieg. Andere Autoren wieder haben keinerlei Veränderung des Blutzuckerspiegels in grösseren Höhen gefunden (*Laubender*, *Wertheimer* u. a.).

*Kreienberg* und *Gerke* (1941) haben initiale Veränderungen des Blutzuckerspiegels bei Versuchen in einer Unterdruckkammer untersucht, bei denen die Herabsetzung des Luftdrucks Höhenlagen von bis 8000 m entsprach. In Abb. 36 ist einer von den Versuchen dieser Forscher wiedergegeben. Aus die-

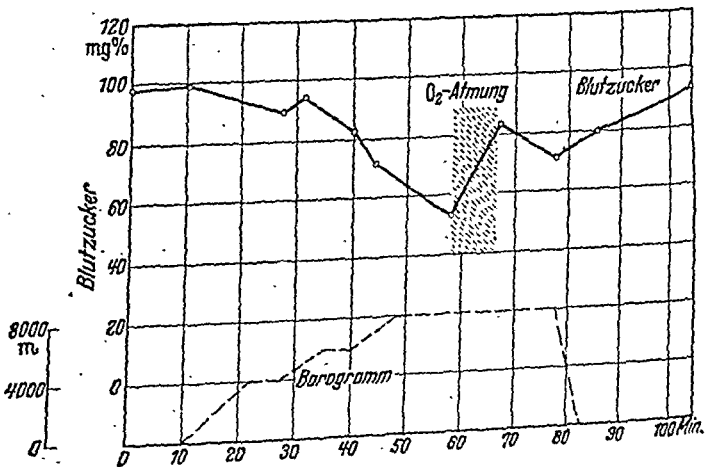


Abb. 36. Veränderung des Blutzuckerspiegels bei herabgesetztem Sauerstoffpartialdruck (Unterdruckkammer, Druckverminderung Höhenlagen von 1 bis 8000 m entsprechend) sowie Wirkung zugeführten Sauerstoffs (nach *Kreienberg*).

sen Untersuchungen geht zweifelfrei hervor, dass Sauerstoffmangel bereits in einem frühen Stadium die Blutzuckerregulation beeinflussen kann.

Bei Sauerstoffmangel und Asphyxie werden auch nervöse Zentren in Mitleidenschaft gezogen. Sie werden anfangs gereizt, aber später gelähmt (*Verworn*). Es ist denkbar, dass eine asphyktische Reizung durch gesteigerte Wirkung des Adrenalin-systems einen Diabetes hervorrufen kann. *Kellaway, Stewart* und *Rogoff* haben indessen gezeigt, dass Hyperglykämie und Zuckerausscheidung bei Erstickung auch nach Entfernung der Nebennieren eintreten. Dagegen scheint der Sauerstoffmangel direkt auf die Leberzellen einzuwirken.

*Lesser, Masing* u. a. haben gefunden, dass ein sauerstoffarmes Perfusionsblut nach der Passage durch überlebende Kaninchenlebern einen höheren Zuckergehalt hat als ein sauerstoffreiches. Dieser Befund spricht entweder für einen gesteigerten Glykogenzerfall oder für eine herabgesetzte Glykogenbildung in der Leber bei mangelnder Sauerstoffzufuhr.

### Der Hungerdiabetes

Ein Diabetes kann ferner bei Hunger oder lange dauerndem Kohlehydratmangel in der Nahrung entstehen. Möglicherweise tritt dabei ein Mangel an denjenigen Intermediärsubstanzen ein, welche zum Fortschreiten der gekoppelten Reaktionen notwendig sind, oder eine Verarmung des Körpers an den die biologischen Oxydationsvorgänge vermittelnden Stoffen. Fehlen Kohlehydrate in der Nahrung, so müssen Glykogensynthese und Zuckerbildung aus Fett oder Eiweiss erfolgen. In erster Linie werden dabei Kohlehydrate aus dem Nahrungsfett oder, wenn solches nicht zur Verfügung steht, aus dem Depotfett im Körper gebildet. Dabei kann sich eine Ketonkörperbildung mit Anhäufung von sauren Stoffwechselprodukten einstellen, was seinerseits zu einer Hungerazidose und auch Hungerglykosurie führen kann.

Der folgende Fall ist ein Beispiel für einen Diabetes vom Hungertyp.

Fall 1. Theodor P., geb. 1878.

Seit August 1933, wo Zucker konstatiert wurde, zeitweise durstig und müde, Pat. magerle nicht ab. Bei Anstrengung Kuzatmigkei mit Oppressionsgefühl. Länge 171 cm, Gewicht 99,0 kg. Pulzfrequenz 100, Blutdruck 170—100. Keine Ödeme, keine Leberschwellung. Herz: Grenzen 3 bzw. 11 cm. Töne dumpf. Keine Arrhythmie. Abb. 37 zeigt Ausscheidungskurven von den Beobachtungstagen 10. II.—14. II. 1935.

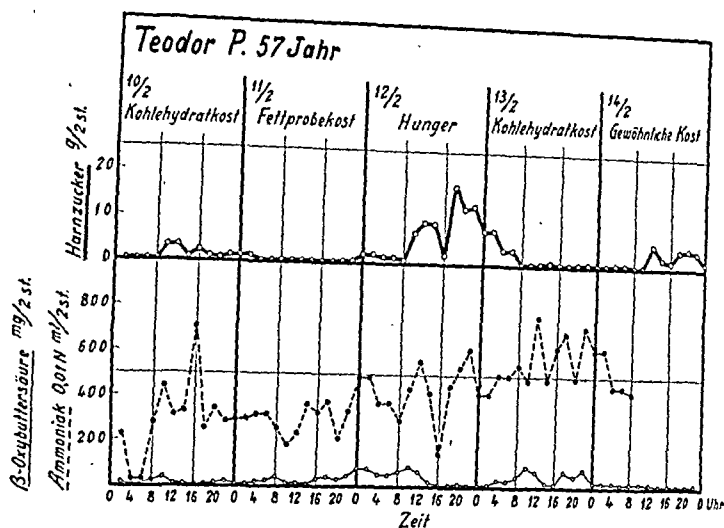


Abb. 37. Fall von Hungerglykosurie bei 47jährigem Diabetiker mit kardialen Beschwerden.

Hier tritt also bei einem fetten Patienten mit Herzveränderungen und versagender Herzkraft während eines Hungertages eine starke Zuckerausscheidung auf. Die Ketonurie, welche sich am Tage vor dem Hungertage bei kohlehydrat- und fettreicher Probekost bemerkbar machte, verschwand mit zunehmender Zuckerausscheidung während des Hungertages.

Nach Elias und Kolb wird ein Hungerdiabetes von der gesteigerten Blut- und Gewebsazidität verursacht. Durch Alkalizufuhr wird die Glykosurie vermindert, umgekehrt steigt sie aber nach Zufuhr kleiner Mengen von Säuren.

Die Injektion von Säuren in die Pfortader steigert nach Pavy und Bywaters den postmortalen Glykogenzerfall in der Leber; Alkali hat die entgegengesetzte Wirkung.

Murlin hat sich mit der Wirkung peroral zugeführter Säure und Alkalikarbonats hinsichtlich des Blutzuckergehalts und der

Glykosurie bei pankreaslosen Tieren beschäftigt. Er fand eine Zunahme des Blutzuckergehalts nach Zufuhr von Mineralsäuren.

*Danell und Underhill* stellten fest, dass eine *basenreiche Ernährung* bei Kaninchen einen höheren Leberglykogengehalt zur Folge hat, als Fütterung mit säurebildenden Nahrungsstoffen. *Eine basenreiche Kost ist daher bei Diabetesformen mit herabgesetzter Leberglykogenbildung indiziert.*

Liegt eine abnorme Produktion organischer Säuren infolge einer Störung des Intermediärstoffwechsels vor, so verschlimmert sich der diabetische Zustand. Dies zeigt sich u. a. in einem gesteigerten Insulinbedarf und in vermehrter Zuckerausscheidung in der ersten Zeit nach dem Verschwinden der Azidose. Hand in Hand mit dem Aufhören der Nachwirkungen der Azidose auf die lebenden Zellen und dem Normalwerden des Säuren-Basengleichgewichts wird der Insulinbedarf Schritt für Schritt kleiner, wobei die Zuckerdiurese sinkt. Die beim Diabetes auftretenden Ketosäuren entstehen bei Störungen im normalen Fettsäureumsatz, wahrscheinlich durch ungenügende Phosphatidbildung (vgl. S. 296). Neben ihrer Bedeutung als Azidose hervorrufender Faktor zeigen daher die Ketosäuren durch ihr Auftreten noch tiefer greifende Störungen im Stoffwechsel an. *Beim Diabetes ist daher in erster Linie eine vorhandene Azidose zu bekämpfen.*

Beim Abbau der Glykose und bei der oxydativen Resynthese des Glykogens tritt mithin *der biologische Oxydationsprozess* in den Vordergrund. Der normale Verlauf desselben ist für den intermediären Kohlehydratstoffwechsel entscheidend. Eine nähere Kenntnis des Verlaufs bei diesem Prozess ist daher eine notwendige Voraussetzung für das Verständnis der normalen und pathologisch veränderten Umsetzung der Kohlehydrate.

### Der biologische Oxydationsprozess

Nur bei hoher Temperatur und mit starken Oxydationsmitteln ist es unter gewöhnlichen Bedingungen möglich, Fette und Kohlehydrate zu den Endprodukten Kohlendioxyd und Wasser

zu verbrennen. Dem lebenden Organismus stehen diese Möglichkeiten nicht zur Verfügung. Der molekulare Sauerstoff, welcher bei der Atmung aufgenommen wird, erreicht an Hämoglobin gebunden Gewebe und Organe. Wenn derselbe wieder frei gemacht wird, vermag er nicht direkt auf das Brennmaterialeinzuwirken, welches aus der verzehrten Nahrung oder aus Depots im Körper stammt und verbraucht werden muss, um den Lebensprozess im Gange zu halten.

Es ist lange ein Rätsel gewesen, wie die biologische Verbrennung zustande kommt. Bei dieser entstehen bei Körpertemperatur Reaktionsprodukte, welche bei einer gewöhnlichen Verbrennung eine so hohe Temperatur erfordern würden, dass auch die Körpergewebe verbrannt werden müssten. Es sind vor allem die grundlegenden Arbeiten von Warburg und von Wieland und Thunberg, denen wir eine teilweise Klarstellung des biologischen Oxydationsvorgangs zu verdanken haben.

Eine Verbrennung organischer Substanzen bedeutet einen Abbau von kohlenstoff- und wasserstoffhaltigen Verbindungen zu einfachen Endprodukten, Kohlendioxyd und Wasser, unter Freimachung von Energie. Hierzu ist die Mitwirkung von Sauerstoff erforderlich.

*Der Oxydationsprozess läuft letzten Endes auf einen Elektronenaustausch zwischen Atomen hinaus.* Dieser Elektronenaustausch wird in der Regel von Sauerstoff- oder Wasserstoffatomen vermittelt. Die Oxydation kann dadurch entweder als eine Aufnahme von Sauerstoff oder als Abgabe von Wasserstoff, eine Dehydrierung, in Erscheinung treten. Auch eine Valenzänderung kann ein oxydativer Vorgang sein.

Die Oxydation einer Substanz ist an die Reduktion einer anderen gekoppelt. Dabei kann die oxydierte Substanz Sauerstoff von der oxydierenden empfangen oder Wasserstoff an dieselbe abgeben.

Molekularer Sauerstoff oder Wasserstoff kann nicht direkt am Oxydationsprozess teilnehmen; die Atome müssen zuerst aktiviert werden. Dies geschieht durch Energiezufuhr in irgendeiner Form, entweder durch direkte Energieaufnahme (z. B. durch Absorption eines Lichtquantums), oder durch Mitwirkung katalytisch aktiver Substanzen.

## Sauerstoffaktivierende Fermente

Die biologische Oxydation erfordert die Mitwirkung einer Reihe von Oxydationsfermenten. Diese Oxydationsfermente lassen sich in zwei verschiedene Gruppen einteilen, die der *sauerstoffaktivierenden* und die der *wasserstoffaktivierenden* bzw. wasserstoffübertragenden Fermente.

Das Vorkommen von Oxydationsenzymen, *Oxydasen*, war bereits 1894 von *Bertrand* u. a. nachgewiesen worden. Die Oxydasen aktivieren molekularen und oxydgebundenen Sauerstoff. Andere Oxydationsenzyme sind die *Peroxydasen*. Diese machen peroxydgebundenen Sauerstoff frei und übertragen denselben auf geeignete oxydierbare Substanzen. *Willstätter* und Mitarbeiter haben zuerst die Isolierung der Peroxydase aus Meerrettich und anderen Pflanzenteilen versucht. *Theorell* stellte 1941 diese häminhaltige Peroxydase in kristallinischer Form dar. *Agner* (1941) konnte aus Leukozyten eine andere häminhaltige Peroxydase, die *Myeloperoxydase*, gewinnen. Dieselbe ist grün und spielt wahrscheinlich bei Infektionszuständen eine wichtige Rolle. Auch in der Milch hat man eine ähnliche Verdoperoxydase die *Lactoperoxydase* gefunden.

Eine weitere Wirkung der Oxydationsfermente ist die *Katalasewirkung*. Dabei wird Wasserstoffsuperoxyd unter Entstehung molekularen Sauerstoffs gespalten. Wahrscheinlich kann der frei gewordene Sauerstoff in statu nascendi zur Oxydation verschiedener Substrate dienen.

Der Sauerstoff kann auch in *Ozonidbindung* auftreten. Diese Bindungsform kommt entweder durch Einwirkung von Ozon auf ungesättigte Kohlenstoffatome zustande, oder durch den Einfluss molekularen Sauerstoffs auf die letzteren, welche durch Absorption von Lichtenergie bei Bestrahlung mit kurzwelligem Licht aktiviert worden sind. Der ozonidgebundene Sauerstoff zerfällt äusserst leicht spontan. Dabei wird aktiver Sauerstoff frei und kann in gewissen Fällen als Oxydationskatalysator wirken (*Swensson* und *Möllerström*).

Der Sauerstoff- bzw. Wasserstoffaktivierung durch die Fermente liegt eine Labilisierung der Elektronenhülle des Atoms durch Elektronenaustausch zwischen Substrat und vermitteln-

dem Enzym zugrunde. Der Elektronenaustausch wird von einem Valenzwechsel oder einer Umlagerung des wirksamen Atoms oder der Atomgruppe im Fermentsystem begleitet. In den *sauerstoffaktivierenden Oxydationsfermenten* sind es in grossem Umfang *Eisen-* oder andere Schwermetallatome, welche Träger der katalytischen Wirkung sind. *Der Wechsel zwischen 2- und 3wertigem Eisen ist das wichtigste Prinzip bei der Sauerstoffaktivierung des biologischen Atmungsprozesses.*

Die Fermentsysteme, welche beim biologischen Oxydationsprozess mitwirken, sind nach einem dualistischen Prinzip aufgebaut, indem ein spezifisch wirkender Eiweisskörper, das *Apoferment*, an eine von demselben meistens reversibel abtrennbare Gruppe, die *prosthetische Gruppe*, auch *Coferment* genannt, gekoppelt ist. *Theorell* hat den ersten experimentellen Nachweis dieses Prinzips erbracht, indem ihm die reversible Spaltung des sog. gelben Oxydationsferments gelang. Apoferment und Coferment bilden zusammen das wirksame Enzymsystem, das *Holoferment*. Das Coferment ist der Träger der oxydo-reduzierbaren Wirkungsgruppe, aber das Apoferment verleiht dem Enzymsystem die spezifische Wirkung desselben. So greift die Cozymase an mehreren Stellen in den Hexo-Triosenabbau ein, wobei sie mit verschiedenen Apofermenten, welche für jede einzelne Reaktion spezifisch sind, zusammenwirkt.

Seiner Natur als Eiweisskörper gemäss kann das Apoferment im Zellprotoplasma verankert sein oder an das Substrat der Wirkung des Enzymsystems spezifisch gebunden werden. Die prosthetische Gruppe dagegen enthält das aktive Atom oder die aktive Atomkonfiguration. Die prosthetische Gruppe wechselt in verschiedenen Fermentsystemen. In gewissen Systemen kann die prosthetische Gruppe sehr rasch zwischen einer oxydierten und einer reduzierten Form hin und her pendeln, wodurch die Fermentwirkung zustandekommt. In anderen Systemen, z. B. bei den Häminproteiden, finden transitorische Anlagerungsprozesse statt, welche die biologische Wirkung zustande bringen.

### Häminproteide und Zytochromsystem

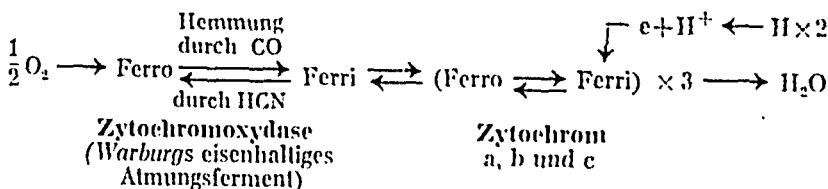
*Warburg* und seine Mitarbeiter untersuchten die Atmung der Hefezellen, der Essigbakterien und der Retina. Diese Atmung

wird von Kohlenoxyd gehemmt. Die Kohlenoxydhemmung des Atmungsferments ist ein reversibler Prozess. Durch Bestrahlung mit Licht wird die Hemmung teilweise aufgehoben. Eine eingehendere Untersuchung des Phänomens ergab, dass das Atmungsferment eine selektive Lichtabsorption besitzt und Farbstoffcharakter haben muss. Weitere Forschungen haben gelehrt, dass das Atmungsferment ein eisenhaltiges Hämiprotein ist.

Das eisenhaltige Atmungsferment ist eine autooxydable Substanz, und die Wirkung desselben beim Oxydationsprozess ist an das Wechseln des Eisenatoms zwischen Zwei- und Dreiwertigkeit gebunden. Ein geringer Sauerstoffdruck genügt bereits dazu, das Fermenteisen restlos von zwei- in dreiwertiges umzuwandeln. Dieser Übergang wird von Kohlenoxyd blockiert, während die Rückbildung zweiwertigen Fermenteisens aus dreiwertigem von Zyanwasserstoff durch Komplexbildung des Eisens gehemmt wird. In beiden Fällen wird die normale zelluläre Atmung verhindert.

Das *Warburgsche eisenhaltige Atmungsferment* oder die *Zytochromoxydase* ist allein für die schliessliche Aktivierung des Sauerstoffatoms nicht ausreichend. Es ist die Mitwirkung von weiteren Hämiproteinen erforderlich, in welchen die Valenz der Eisenatome reversibel zwischen Zwei- und Dreiwertigkeit wechseln kann. Diese Hämiproteine sind an Gewebe gebunden und entsprechen dem von *Mac Munn* entdeckten Histohämatin. Sie können von Sauerstoff nicht direkt oxydiert werden, wohl aber sukzessive durch Vermittelung des eisenhaltigen Atmungsferments. Die sauerstoffübertragende Wirkung derselben wird von Kohlenoxyd oder Zyanwasserstoff nicht aufgehoben.

Der Mechanismus der Sauerstoffaktivierung lässt sich rein schematisch folgendermassen darstellen:

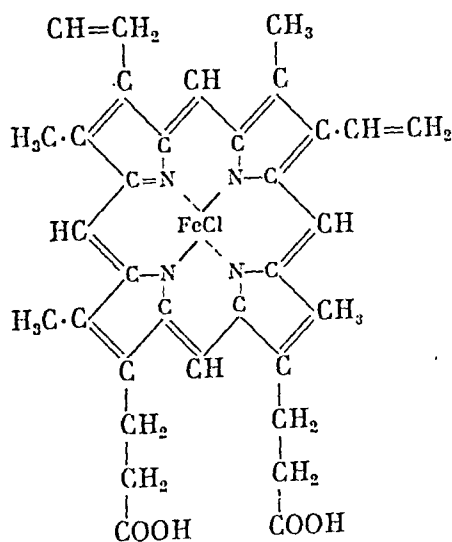


Die drei Histohämiproteine sind an Zellsubstanzen gebunden. Auf Grund ihres Farbstoffcharakters werden sie nach



*Keilin* als Zytochrom a, b und c bezeichnet. Sie vermitteln den Elektronenaustausch zwischen dem aus den Substraten herstammenden Wasserstoff und dem molekularen Sauerstoff. Dabei wird Wasser gebildet. Im Gegensatz zu dem diesbezüglichen Verhalten des Atmungsferments wird im Zytochromsystem der Valenzwechsel der Eisenatome nicht von Zyanwasserstoff oder Kohlenoxyd verhindert.

Die prosthetischen Gruppen in den sauerstoffaktivierenden Fermentsystemen lassen sich sämtlich aus dem *Hämin* herleiten. Das Hämin wird von vier Pyrrolkernen aufgebaut, welche durch Methingruppen zu einem ringförmigen Molekül mit einem zentralen Eisenatom zusammengekoppelt werden. Neben vier Methylgruppen enthält das Protohämin als Seitenketten zwei Vinylgruppen und zwei Propionsäuregruppen. Von den zahlreichen isomeren Möglichkeiten ist nach *Fischer* die dem Protohämin IX entsprechende Konfiguration die biologisch wichtigste.



**Protohämin IX**

Im Zytochrom b scheint die prosthetische Gruppe aus Protohämin zu bestehen. Dies ist auch in der Katalase und in der Meerrettichperoxydase der Fall.

Im Zytochrom c sind dagegen die Vinylgruppen des Protohä-

mins nach *Theorell* durch Addition der SH-Gruppen zweier Zysteinmoleküle gesättigt, welche die Verankerung des Zytchroms c an die Eiweisskomponente vermitteln. Das wirk-same Eisenatom selbst ist dabei durch zwei Imidoazolgruppen fest mit dem Eiweisskomplex verbunden. Der Valenzwechsel desselben bewirkt eine reversible Bindungsverschiebung im Komplex.

*Ein intaktes Ferment- und Zytchromsystem ist die Grundbedingung für den normalen Verlauf der Oxydation.*

## Wasserstoffaktivierende und wasserstoffübertragende Fermente

Auf die Bedeutung der Dehydrierung für die biologische Oxydation hat als erster *Wieland* aufmerksam gemacht. Durch die Wirkung dehydrierender Enzyme, der *Dehydrasen*, werden Wasserstoffatome mobilisiert. Hierdurch wird die Übertragung derselben auf ein aktiviertes Sauerstoffatom oder eine andere wasserstoffaufnehmende Substanz vorbereitet.

Von der *Wielandschen* Dehydrierungstheorie ausgehend haben *Thunberg* und dessen Mitarbeiter die Oxydation organischer Säuren im sauerstofffreien Medium bei Anwesenheit von geeigneten Wasserstoffakzeptoren, z. B. Methylenblau, untersucht. Sie haben spezifisch wirkende Dehydrasen gefunden, welche sich an der Oxydation von Bernsteinsäure, Milchsäure, Apfelsäure, Zitronensäure, Glutaminsäure und anderen Substanzen beteiligen.

Beim oxydativen Abbau der Kohlehydrate ist die Mitwirkung von mindestens zwei wasserstoffaktivierenden Fermentsystemen bei der Übertragung des Wasserstoffs erforderlich. Diese wasserstoffübertragenden Fermente können mit Rücksicht auf den Bau der prosthetischen Gruppe unter die Alloxazinproteide und die Pyridinproteide eingereiht werden.

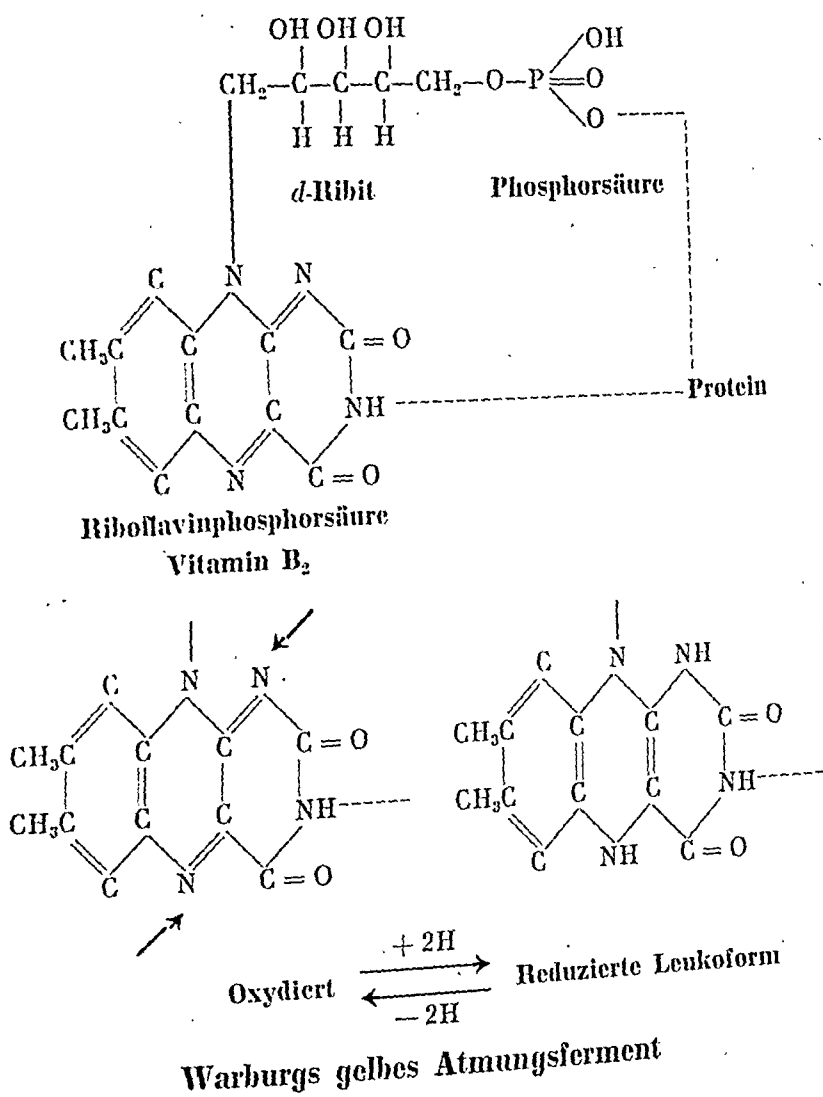
## Die Alloxazinproteide

### *Gelbes Atmungsferment und Vitamin B<sub>2</sub>*

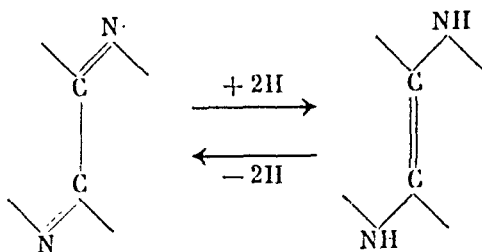
Das von *Warburg* und *Christian* 1932 entdeckte gelbe Atmungsferment ist ein Alloxazinprotein. Der Bau desselben

ist durch Arbeiten von *Warburg*, *Kuhn*, *Karrer* und *Theorell* klargestellt. Die prosthetische Gruppe im gelben Atmungsferment wird von einem heterozyklischen Ringsystem und einer Kohlehydratkomponente, dem Pentit *d*-Ribit, aufgebaut; sie bildet Riboflavin, welches mit Vitamin B<sub>2</sub> identisch ist. Das Riboflavin muss an Phosphorsäure gekoppelt werden, um seine Wirkung entfalten zu können. Nach *Theorell* vermittelt die Phosphorsäure die Bindung an den spezifischen Eiweisskörper, welcher in das gelbe Atmungsferment eingeht.

Nach *Kuhn* und *Rudy* ist die Konstitution des gelben Atmungsferments folgende:



Die Fähigkeit des gelben Atmungsferments, bei dem biologischen Oxydationsprozess Wasserstoff aufzunehmen und abzugeben, ist an die Atomkonfiguration



gebunden.

Hier tritt keine Valenzänderung der wirksamen Stickstoffatome ein, sondern nur eine zur Entstehung einer Doppelbindung zwischen zwei Kohlenstoffatomen führende Bindungsverschiebung mit daraus folgenden energetischen Spannungszuständen in der Kohlenstoffkette.

### Flavin-Adeninnukleotide

Karrer und seine Mitarbeiter haben in der Leber eine Flavinphosphorsäureverbindung gefunden, welche ausserdem Adenin enthält. Nach Straub, Warburg und Christian ist dieses Flavin-Adeninnukleotid nach Koppelung an ein spezifisches Fermentprotein mit einer von Krebs gefundenen, bei der oxydativen Desaminierung der Aminosäuren wirksamen Aminosäuren-oxydase identisch. Die prosthetische Gruppe wird hier von Riboflavinphosphorsäure + Adenylsäure unter Austritt eines Moleküls  $\text{H}_2\text{O}$  gebildet. Diese prosthetische Gruppe, an ein anderes spezifisches Protein gebunden, ist nach Ball mit der Xanthinoxidase identisch, welche bei der Oxydation des Hypoxanthins zu Harnsäure (s. S. 107) in Tätigkeit tritt.

Das Vitamin  $\text{B}_2$  spielt also beim Aufbau der prosthetischen Gruppe in den wasserstoffübertragenden Alloxazinproteiden eine bedeutende Rolle, welche bei den Oxydationsprozessen im Organismus als Atmungsfermente wirksam sind.

Bevor das Riboflavin zum Aufbau des Atmungsferments ver-

wendbar ist, muss dasselbe an Phosphorsäure gekoppelt werden. Der Phosphorylierungsprozess geht wahrscheinlich schon in der Darmwand vonstatten.

Die aus dem Darm resorbierte Riboflavinphosphorsäure sammelt sich dann in der Leber an. Sie wird an spezifische Eiweißkörper gebunden, wobei wirksame Atmungsfermente entstehen.

Der Wasserstofftransport der Alloxazinproteide ermöglicht die Bildung von  $H_2O$  durch Verbindung des Wasserstoffs mit dem durch das Zytochromsystem aktivierten Sauerstoff.

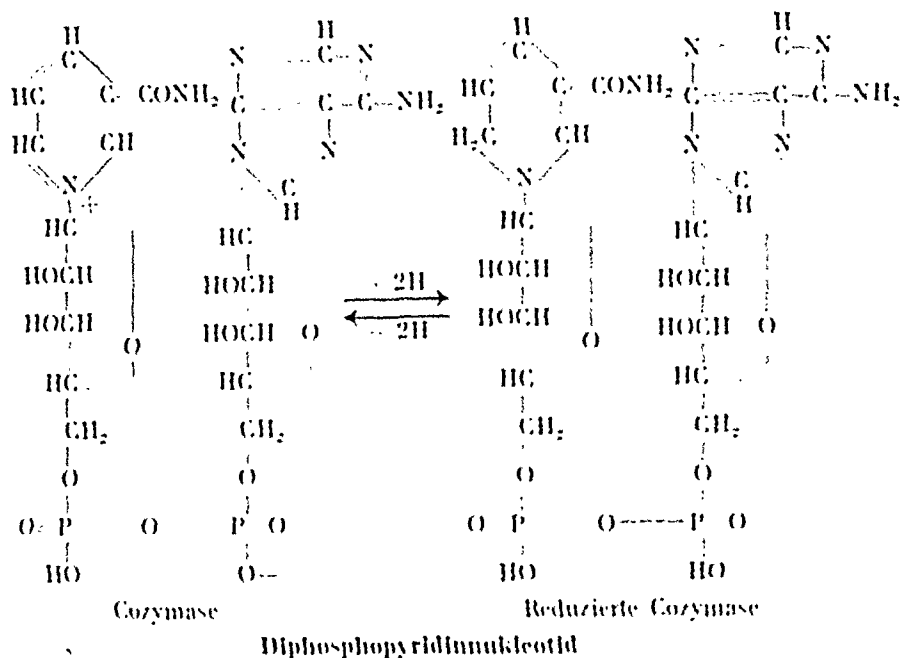
### Die Pyridinproteide und das wasserstoffübertragende Zwischenferment

Die Freimachung des Wasserstoffs aus dem Kohlenstoffgerüst des Brennmaterials macht die Mitwirkung eines weiteren Fermentsystems erforderlich, welches ebenfalls von einer prosthetischen Gruppe und einem spezifischen Protein aufgebaut wird. Der Wirkungsfaktor der prosthetischen Gruppe ist hier nach Warburg ein Pyridinderivat, das Nikotinsäureamid. Die Proteinkomponente und die prosthetische Gruppe befinden sich durch reversible Bindung im Dissoziationsgleichgewicht. Hierdurch ist es möglich, die Eiweißkomponente von der prosthetischen Gruppe zu trennen. Diese ist dialysabel, thermostabil und stimmt mit der seit den grundlegenden Dialyseversuchen von Harden und Young (1905) mit Hefepresssaft bekannten Cozymase überein. Durch Arbeiten von Euler, Myrbäck und Nilsson wurde schon früh (1926) erwiesen, dass die Cozymase ein notwendiger Aktivator für die enzymatische Dehydrierung ist.

Aus roten Blutkörperchen konnten Warburg und Christian 1934 ein dehydrierendes Coferment von ähnlicher Art wie die Cozymase darstellen, welches eine Pyridinbase, das Nikotinsäureamid, enthält. Die Cozymase und das Warburgsche wasserstoffübertragende Coferment sind beide Codehydrasen und aus Adenin, Nikotinsäureamid, zwei Pentosemolekülen (*d*-Ribose), aber mit zwei bzw. drei Phosphorsäuremolekülen aufgebaut. Die Cozymase ist folglich ein *Diphosphopyridinnukleo-*

tid, das Warburgsche wasserstoffübertragende Coferment dagegen ein Triphosphopyridinnukleotid.

Der Bau der Cozymase ist durch Arbeiten von u. a. Schlenk dargestellt und wird in der hier folgenden Strukturformel



wiedergegeben. Die Cozymase baut sich aus einem Adeninnukleotid und einem Pyridinnukleotid auf.

Die wasserstoffübertragende Funktion des Pyridins ist an einen reversiblen Oxydo-Reduktionsprozess gebunden. Dabei wird wahrscheinlich eine intramolekulare Salzbindung gelöst. Ein Elektronenaustausch mit zwei Wasserstoffatomen, welche an den betreffenden Pyridinkern und an die Phosphorsäure des Adeninnukleotids gebunden werden, kann stattfinden. Dann kann eine reversible Spaltung der reduzierten Form der Codehydrase erfolgen. Neben dem Pyridinnukleotid wird da auch Adenosin bzw. Adenylsäure gebildet, welche Phosphorsäure unter Entstehung von Adenosintriphosphorsäure binden können. Diese kann wiederum Phosphorsäure an die Veresterungsform des Hexosemoleküls abgeben, evtl. durch Ver-

mittlung des Phosphagens (S. 108). Da der Phosphorylierungsprozess den Hexoseabbau einleitet, ist wahrscheinlich bereits die Phosphorylierung selbst an die Wirkung der Atmungsfermente und die biologische Oxydation in der lebenden Zelle gekoppelt.

Das Pyridinnukleotid kann wieder durch intermediär beim Kohlehydratumsatz gebildeten Azetaldehyd oder Brenztraubensäure oxydiert werden. Dabei kann der Codehydrasekomplex aus seinen Bruchstücken wiederentstehen.

Durch den Einfluss von im sowohl Tier- wie Pflanzenreich verbreiteten Enzymen, der Nuklease u. a., können die Cofermente in ihre Komponenten gespalten werden, wobei die Wirkung erlischt.

Der sich ständig wiederholende Abbau von Kohlehydraten ist somit eine Voraussetzung für die Neubildung der dehydrierenden Cofermente. Wie *Lennerstrand* hervorgehoben hat, müssen in der lebenden Zelle codehydrasebildende Prozesse das Übergewicht haben, sonst verschwindet die Codehydrasewirkung, Phosphorylierungs- und Dehydrierungsvorgänge machen halt, wobei der normale Kohlehydratabbau aufhört. In der lebenden Zelle herrscht ein dynamisches Gleichgewicht zwischen Energie erfordernden synthetischen Prozessen auf der einen und einem spontan verlaufenden enzymatischen Abbau auf der anderen Seite.

Bildung und Funktion der Codehydrasen sind also einmal von der Energieerzeugung des Kohlehydratstoffwechsels abhängig, sodann von dem Nukleasesystem, welches das Pyridinnukleotid und den Codehydrasekomplex abbaut. Verläuft die Zuckerumsetzung normal, so wird nach *Lennerstrand* das dynamische Gleichgewicht in einem System: Adenosin + Nikotinsäureamid + Phosphorsäure in der Richtung auf eine vermehrte Bildung von Codehydrase verschoben. Diese kann dann als wasserstoffübertragender Faktor wirksam zur Erhaltung des zellulären Atmungsprozesses und dadurch zum normalen Umsatz der Kohlehydrate in der Zelle beitragen. Wenn dagegen der Zuckerabbau gehemmt wird, nimmt das Nukleasesystem überhand. Da wird die Codehydrase abgebaut und es scheinen auch Adenin, Adenosin, Nikotinsäureamid und Phosphorsäure

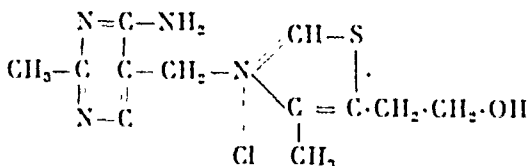
nach *Lennerstrand* frei zu werden. Die Codhydrasesynthese kann dabei nicht durch vermehrte Zufuhr dieser Substanzen gesteigert werden. Die Dehydrasewirkung wird abgeschwächt, und die zellulären Prozesse kommen trotz erhöhter Nahrungszufuhr zum Stehen. Es tritt hierbei eine Störung im normalen Kohlehydratabbau ein, welche zu einem Diabetes führen kann.

Die Ausscheidung von Pyridinderivaten kann ein Ausdruck für den endogenen Pyridinumsatz sein. Beim Diabetes lassen sich auch deutliche Schwankungen erkennen, und in gewissen Fällen ein 24stundenperiodischer Verlauf mit einem Minimum in der Nacht und zwei Maxima am Tage, eins etwa um 10—12 Uhr und eins etwa um 18—20 Uhr (vgl. S. 314).

Es ist bemerkenswert, dass die Pyridinausscheidung bei gewissen Fällen von Diabetes bei fettreicher Kost geringer ist als bei kohlehydratreicher, weshalb Fette den endogenen Pyridinstoffwechsel weniger zu belasten scheinen als den Kohlenhydratumsatz (vgl. S. 316).

### Dekarboxylierende Fermente und Vitamin B<sub>1</sub>

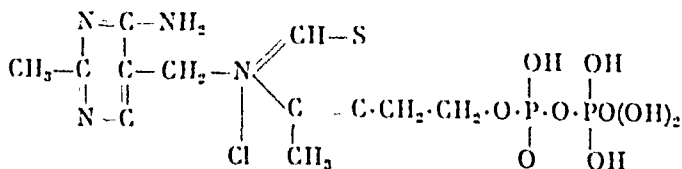
Bei der Verbrennung im Organismus entsteht neben Wasser Kohlensäure. Diese kommt durch Dekarboxylierungsprozesse



Dimethylaminopyrimidin

Methyloxyäthylthiazol

#### Aneurinchlorid



Aneurin

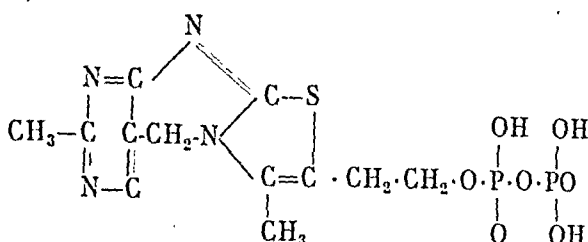
Pyrophosphorsäure

#### Vitamin B<sub>1</sub>



zustande. Auch bei diesem Vorgang ist nach *Auhagen* die Mitwirkung eines Coferments erforderlich. Diese Cokarboxylase wurde 1937 von *Lohmann* und *Schuster* rein dargestellt. Sie ist ein pyrophosphoryliertes Aneurin. Aneurin ist ein Pyrimidin-Thiazolderivat. Die Konstitution desselben ist von *Williams*, *Windaus* und *Grewe* erforscht worden.

Bei vorsichtiger Oxydation geht das Aneurin in das stark fluoreszierende Thiochrom über. Hierbei erfolgt nach *Todd* ein Ringschluss zwischen der Aminogruppe und dem angrenzenden Kohlenstoffatom im Thiazolkern. Dabei verschwindet die Wirkung des Vitamins B<sub>1</sub>.



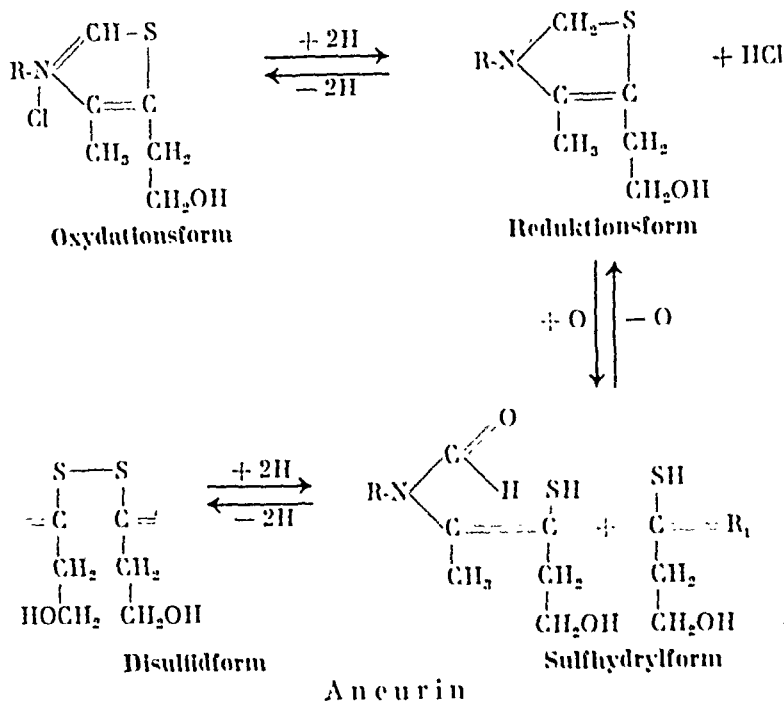
**Thiochrom**

*Kuhn* und *Wetter*, *Lipmann* u. a. haben gefunden, dass das Aneurin bei Reduktion Wasserstoff aufnimmt. *Lipmann* und *Perlmann* wiesen nach, dass sich diese Reduktion in der Thiazolkomponente abspielt, nicht im Pyrimidinrest.

Nach *Lipmann* ist die Wirkung des Aneurins an eine reversible Oxydo-Reduktion gebunden, durch welche die Oxydationsform in die Reduktionsform übergeht, wobei ein Wasserstoffatom aktiviert werden soll. Der Wirkungsmechanismus des Aneurins ist aber noch nicht endgültig geklärt. Das Vitamin B<sub>1</sub> ist jedenfalls ein wirksamer Faktor bei der Freimachung des Kohlendioxyds im Verlauf der intermediären Stoffwechselprozesse, speziell bei der Dekarboxylierung der Brenztraubensäure.

Das Aneurin kann wahrscheinlich auch als wasserstoffübertragender Faktor dienen.

*Zima* und *Williams* haben nämlich gefunden, dass unter dem Einfluss von Alkali der Thiazolring des Aneurins gesprengt wird, wobei als Oxydationsprodukt ein Aneurindisulfid entsteht, welches sie in kristallinischer Form dargestellt haben. Durch



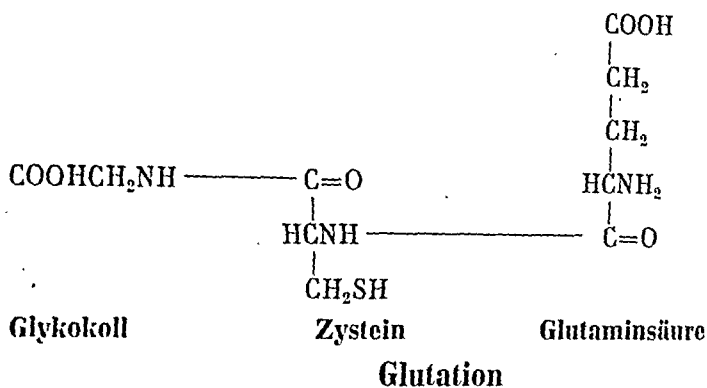
Reduktion kann nach Zima das Aneurin aus der Disulfidform auch in die Sulphydrylform übergehen. Durch erneute Oxydation kann diese Sulphydrylform in die Disulfidform zurückgehen oder in die ursprüngliche Form weiterumgewandelt werden. Ob dieses Aneurindisulfid im Organismus biologische Bedeutung für die Oxydationsprozesse besitzt, ist noch nicht mit Sicherheit bekannt.

### Andere biologisch wichtige reversible Oxydo-Reduktionssysteme

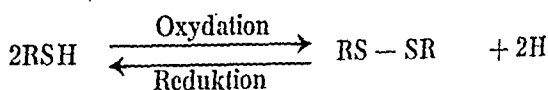
Die reversiblen Redoxsysteme spielen eine wichtige Rolle als physiologisch-chemische Regulatoren der Dehydrierungsprozesse und steuern dadurch den biologischen Atmungsvorgang in den lebenden Zellen. Sie greifen teils direkt in Oxydations- und Reduktionsprozesse des Organismus ein, teils aktivieren oder hemmen sie die Wirkung anderer Fermentsysteme, welche bei der biologischen Oxydation tätig sind.

## Glutathion

Ausser den vorstehend besprochenen Atmungsfermenten gibt es noch andere für den Lebensprozess wichtige Redoxsysteme. Ein solches von mehr historischen Interesse ist das von *Hopkins* 1921 entdeckte *Glutathionsystem*. Das Glutathion ist ein in Eiweisskörpern aus verschiedenen Organen des Körpers vorkommendes schwefelhaltiges Tripeptid, welches aus Glykokoll, Zystein und Glutaminsäure unter Wasseraustritt aufgebaut wird.



Der Oxydo-Reduktionsprozess ist hier mit der wechselnden Bindung des Schwefelatoms in Sulfhydryl- oder Disulfidform verknüpft.

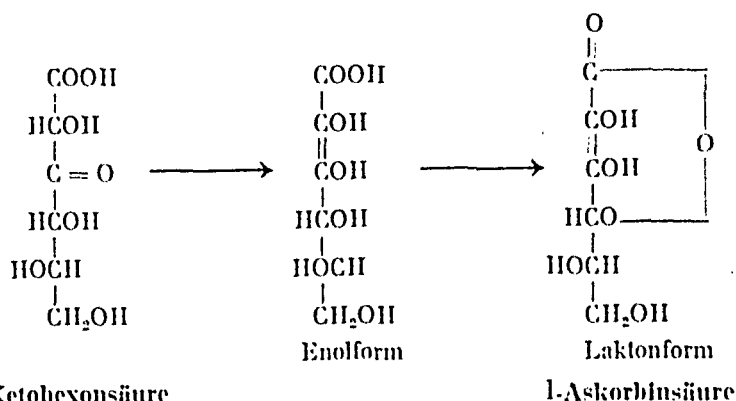


## Vitamin C

Ein anderes reversibles Redoxsystem ist die *Ascorbinsäure* oder das *Vitamin C*.

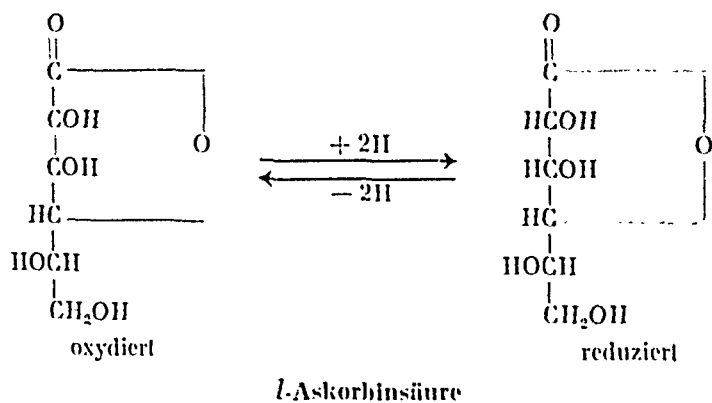
Durch die grundlegenden Untersuchungen von *Holst* und *Fröhlich* über den Meerschweinchenskorbut wurde endgültig erwiesen, dass diese Erkrankung von Mangel an einer Substanz verursacht wird, welche auf Vorschlag von *Drummond* (1920) später als *Vitamin C* bezeichnet worden ist. Durch Arbeiten von *Szent-Györgyi* wurde 1931 klargestellt, dass dieses Vitamin mit der stark reduzierenden Hexuronsäure identisch ist. Drei Jahre vorher hatte *Szent-Györgyi* diese Säure aus Nebennieren

dargestellt. Die Konstitution der Hexuronsäure oder, wie sie in der Folge genannt wurde, Askorbinsäure ist aus Arbeiten von *Szent-Györgyi*, *Haworth* und *Hirst* bekannt. Sie ist die Lakt-onform einer enoltransformierten 3-Ketohexonsäure.



### Vitamin C

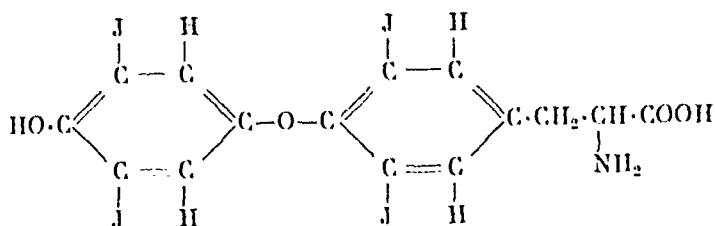
Die Askorbinsäure zeichnet sich durch ein starkes Reduktionsvermögen aus; sie nimmt dabei Wasserstoff auf und geht in die reduzierte Form über.



Durch schwächere Oxydation kehrt sie reversibel zu ihrer ursprünglichen Form zurück. In saurer Lösung ist die Askorbinsäure stabil.

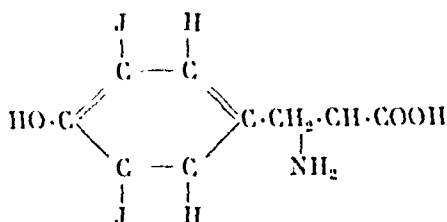
Bei stärkerer Oxydation und in alkalischer Lösung geht die Oxydation des Vitamins C bis zur Bildung der inaktiven De-





Thyroxin

Neben dem Thyroxin enthält das Thyreoglobulin noch Dijodthyrosin.



Dijodthyrosin

Das Dijodthyrosin hat ähnliche, wenn auch schwächere, Wirkungen wie das Thyroxin. Bei Hyperthyreose scheint das Dijodthyrosin nach *Abelin* die Thyroxinwirkung zu dämpfen.

Nach *Schilddrüsenexstirpation* sinkt der Grundumsatz. Dies scheint auf einer Beschränkung der Oxydationsprozesse in den peripheren Geweben zu beruhen. Da gleichzeitig die Ausscheidung von sowohl Harnstoff wie Kreatin sinkt, ist es hauptsächlich die Eiweissverbrennung im Organismus, welche vermindert wird. Der Kohlehydratstoffwechsel wird dagegen nicht in entsprechendem Grade beeinflusst, und der Glykogengehalt der Muskulatur und der Leber ist nach Schilddrüsenentfernung unverändert. Der Fett- und Lipoidgehalt des Blutes steigt, und bei mangelhafter Schilddrüsenfunktion besteht eine Tendenz zur Fettdegeneration im Organismus.

Das Thyroxin setzt den Glykogengehalt der Leber herab (vgl. S. 46). Bei normalen Tieren beginnt jedoch der Glykogengehalt der Leber bei Thyroxinbehandlung nach einigen Tagen wieder zu steigen, und gleichzeitig nimmt der Sauerstoffgrundverbrauch zu. Nach *Cramer* (1928) löst Thyroxin neben einem Glykogenzerfall noch eine Gegenreaktion aus, welche zu einer gesteigerten Glykogenbildung in der Leber führt. *Stern-*

heimer (1929) fand, dass der anfänglichen Glykogenverminderung bei der Thyroxinwirkung eine vorübergehende Eiweissanhäufung in der Leber folgte, welche ihrerseits nachliess, wenn das Glykogen wieder zuzunehmen begann. Das Thyroxin beschleunigt demnach auch die Glykogenbildung aus Eiweiss.

Abelin und Althaus (1942) haben gezeigt, dass diese Glykogenbildung aus Eiweiss von der Funktion der Nebennierenrinde abhängig ist und mit einer vom Thyroxin ausgelösten vermehrten Cortinbildung zusammenhängt. In bezug auf das Leberglykogen scheinen Thyroxin und Cortin einander in einem hormonalen Gleichgewicht zu halten, bei dem der Glykogenzerfall auf der einen, die Glykogenneubildung aus Eiweiss auf der anderen Seite von der Thyroxin- bzw. Cortinwirkung geregelt wird.

Der Sauerstoffgrundverbrauch nimmt nach Thyreoglobulin stärker zu als nach Thyroxin (Abb. 38). Der gesteigerte Sauerstoffverbrauch tritt ein, nachdem die Glykogenbildung aus Eiweiss in Gang gekommen ist. Da kann der Glykogengehalt der Leber trotz eines lebhafteren Glykogenzerfalls infolge der Thyroxinwirkung wieder normal werden.

Bei Unterfunktion der Thyreoidea kommt es auch zu Störungen der normalen Wärmeregulation des Körpers und zu abnormer Empfindlichkeit gegen Abkühlung und sinkende Körpertemperatur. Dies wird nach v. Issekutz wahrscheinlich durch einen Einfluss auf das Wärmeregulationszentrum verursacht.

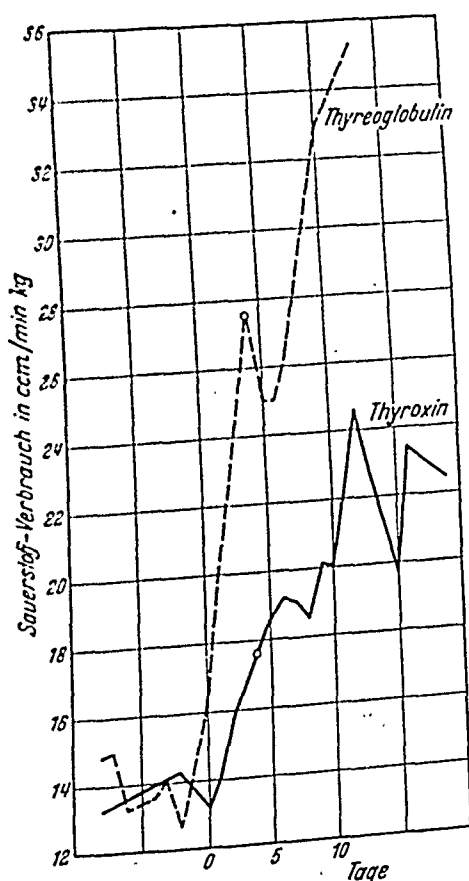


Abb. 38. Sauerstoffverbrauch nach peroraler Zufuhr von Thyreoglobulin und Thyroxin, 2,6 mg Jod/kg (nach Gaddum).

## VI. ABSCHNITT

## Die neurohormonale Regulation des Kohlehydratstoffwechsels.

Die den Lebensprozess begleitenden chemischen Umsetzungen sind in grossem Umfang an eine Kohlehydratverbrennung in den lebenden Zellen gekoppelt. Es muss daher im Organismus stets Blutzucker in hinreichender Menge zur Verfügung stehen. Wird der Blutzuckergehalt zu niedrig, so wird die Umsetzung ungenügend, die Energiequelle droht zu versiegen, und es kommt zu einem das Leben gefährdenden hypoglykämischen Zustand. Ist dagegen der Blutzuckergehalt zu hoch, so wird die Glykose nicht ausgenützt und der Überschuss vom Organismus ausgeschieden. Der Blutzuckerspiegel darf demzufolge normalerweise nur innerhalb enger Grenzen variieren: im Hunger 80—120 mg%, bei Nahrungszufuhr mit vorübergehenden Steigerungen bis zu 180 mg%, und entsprechend der endogenen Rhythmik des Zuckerstoffwechsels etwas schwankend.

## Einwirkung verschiedener regulatorischer Faktoren auf den Blutzuckergehalt

Für das Konstantbleiben des Blutzuckergehalts sorgt die Blutzuckerregulation, indem sie das Gleichgewicht zwischen blutzuckersenkenden und -steigernden Faktoren herstellt. Diese Faktoren müssen bei normaler Blutzuckerregulation harmonisch koordiniert sein. Hierbei greift das Nervensystem auf mannigfache Weise durch von vegetativen, stoffwechselregulierenden Zentren in den basalen Hirnganglien, evtl. auch von anderen Teilen des Gehirns, ausgehende direkte oder reflektorische





Bei normaler Blutzuckerregulation wird der Effekt der wechselnden Kohlehydrataffinität der Leber zum grossen Teil von anderen regulatorischen Faktoren ausgeglichen, und deshalb ist der Blutzuckergehalt normalerweise relativ konstant. Bei gewissen Fällen von Diabetes dagegen, mit Störung der Blutzuckerregulation aber verhältnismässig normaler Glykogenbildung in der Leber, können die spontanen Blutzuckerfluktuationen im Zusammenhang mit der rhythmischen Lebertätigkeit sehr deutlich hervortreten.

3. Einen dritten blutzuckerregulierenden Faktor stellen die *Resorptionsverhältnisse im Darmkanal* dar.

Eine Voraussetzung für die Verwertung von Kohlehydraten ist der Abbau von Polysacchariden zu einfachen Zuckerarten durch die Verdauungsarbeit. Für die Resorption ist die Funktion der Darmschleimhaut ausschlaggebend, und diese steht u. a. unter hormonalem Einfluss seitens der Hypophyse und der Nebennieren (s. S. 79).

4. Ein für die Blutzuckerregulation wichtiger Faktor ist die *Nierenfunktion*. Diese dient zwei prinzipiell verschiedenen Arbeitsaufgaben, einmal der *Ausscheidungsfunktion* der Niere, sodann der *Mitwirkung derselben beim Zwischenstoffwechsel*. Die Niere wird von grossen Blutmengen durchströmt, und das Nierenparenchym verbraucht Sauerstoff in reichlicher Masse, wodurch wesentliche Voraussetzungen für ein aktives Eingreifen des Organs in den oxydativen Stoffwechsel geschaffen werden.

Schon *Claude Bernard* hatte gefunden, dass die Harnausscheidung von nervösen Einflüssen abhängig ist. Werden die Gefässnerven der Niere am Hilus durchtrennt, so erfolgt eine Vasodilatation mit Hyperämie in der Niere und vermehrter Urinausscheidung. Senkt man dagegen den Blutdruck durch Vagusreizung, so nimmt auch die Harnmenge ab.

Die Harnbildung findet wahrscheinlich durch einen kombinierten Filtrationsprozess in den Glomeruli mit Rückresorption gewisser Substanzen in den Nierenkanälchen, namentlich in den *Henleschen Schleifen* und *Tubuli contorti*, statt (*Ludwig-Cushny-Rehbergsche* Theorie der Harnbildung). Die biologische Permeabilität der Niere ist daher einerseits von einem Filtrationsprozess und andererseits von einer Rückresorption abhän-

sig; dadurch beruht die Harnausscheidung in hohem Grade auf der Aktivität des Nierenepithels. Die von *Claude Bernard* aufgestellte Theorie von der Nierenschwelle, bei welcher der Niere die Rolle eines passiv wirkenden Organs zugeschrieben wird, ist daher bezüglich ihrer Geltung umstritten. Für die Zuckerausscheidung stellt die Nierenschwelle ebenfalls einen variablen Faktor dar.

*Chabanier, Speranza* u. a. haben gefunden, dass der Schwellenwert nicht nur individuell, sondern auch bei ein und derselben Person an verschiedenen Zeitpunkten variiert. *Rabinowitch* beobachtete einen Diabetesfall, bei dem der Blutzuckergehalt das eine Mal ohne Glykosurie auf 0,40 % steigen konnte; bei anderen Gelegenheiten trat dagegen schon bei einem Blutzuckergehalt von 0,16 % eine erhebliche Zuckerausscheidung auf. Hier lag wahrscheinlich eine pathologisch veränderte Nierenfunktion vor.

Auch bei normaler Nierenfunktion besteht keine einfache Beziehung zwischen Glykämie und Glykosurie. *May* liess neun Versuchspersonen, welche nach 50 g Glykose die normale glykämische Reaktion aufwiesen, 1000 g Glykose einnehmen, worauf Blut- und Harnzuckergehalt laufend untersucht wurden. Fünf von diesen Versuchspersonen reagierten mit Hyperglykämie und Glykosurie; die vier übrigen bekamen keine Hyperglykämie, aber eine derselben schied bis 2 % Zucker im Urin aus. Bei sieben der Versuchspersonen hielt die Glykosurie bis 18 Stunden nach der Erreichung normaler Blutzuckerwerte an. Nach *May* soll die Niere normalerweise überschüssigen Zucker auch ohne vorangehende Hyperglykämie eliminieren können.

*Eisner* und *Forster* meinen, es herrsche ein Parallelismus gewissen Grades, aber keine gesetzmässige Beziehung, zwischen Blutzuckergehalt und Glykosurie.

Wie andere lebende Zellen nehmen auch die Nierenzellen Glykose auf, welche verbrannt wird. In der Norm findet jedoch im Nierenparenchym keine Glykogenspeicherung statt. Beim Diabetes hingegen fand schon *Ehrlich* in vielen Fällen eine Glykogenbildung im Nierenepithel. Dieses Glykogen tritt

stets mit einer bestimmten Lokalisation im Nierenparenchym auf, und zwar an der Grenze zwischen Mark und Rinde.

Nach *Eisner* und *Forster* sollen bei Hyperglykämie abnorme Glykogenmengen in Geweben und Organen, und damit auch in den Nieren, gespeichert werden. Sekundär zu dieser Glykogenablagerung in den Nieren entstehe nach *Eisner* und *Forster* die Glykosurie. Es lässt sich indessen schwer entscheiden, ob die Glykosurie das Sekundäre, eine Glykogenablagerung im Nierenepithel das Primäre ist, oder ob die Glykogenbildung die sekundäre Folge einer Zuckerdiurese darstellt, und zwar durch eine Rückresorption des Zuckers, welcher im Glomerulusharn aus dem Blute ausgeschieden wird. Die Lokalisation des Nierenglykogens in den Tubuli contorti deutet darauf hin, dass die Glykogenbildung in den Nieren sich sekundär zu einer Rückresorption von Glykose verhalten kann, und gäbe in diesem Falle eine Stütze für die *Ludwig-Cushnysche* Theorie ab.

Schon bei der normalen Harnbildung findet eine Rückresorption von Glykose in den Nierenkanälchen statt. Diese Rückresorption setzt nach *Lundsgaard* und *Kalckar* eine Phosphorylierung der Glykose voraus, einen Prozess, welcher mit der zellulären Atmung des Nierenepithels verknüpft ist. Bleibt die Phosphorylierung aus, so kann die Glykose nicht rückresorbiert werden, und sie verlässt da den Körper mit dem Urin.

*Lundsgaard* hat nachgewiesen, dass die Phloridzinvergiftung eine Störung der Phosphorylierung bewirkt, bei der die renale Rückresorption der Glykose verhindert wird. Es entsteht da ein Diabetes vom renalen Typus. Der intermediäre Kohlehydratstoffwechsel im Organismus braucht dabei sonst nicht gestört zu sein.

Die Rückresorption der Glykose ist für die Höhe der Nierenschwelle ausschlaggebend. Alle Momente, welche die Rückresorption beeinflussen, verändern demnach die Nierenschwelle. Unter den hierbei wirksamen Faktoren steht die Funktion der Hypophyse — direkt oder indirekt — an wichtiger Stelle. *Lucke* (1934), *Russell* und *Cori* haben durch Tierversuche gezeigt, dass die Zuckerausscheidung nach Hypophysektomie auch dann zurückgeht, wenn der Blutzuckergehalt zunimmt.

Jede Glykosurie setzt eine funktionell veränderte Nierentätig-

keit voraus. Die eine Hyperglykämie auslösenden Faktoren sind daher von denjenigen zu unterscheiden, welche die Glykosurie bewirken. Die Funktion der Niere muss bei einer normalen Blutzuckerregulation mit den übrigen glykoregulatorischen Faktoren im Einklang stehen.

5. Ein fünfter Faktor bei der Regulation des Blutzuckers sind der oxydo-reduktive Zuckerabbau im Organismus und die mit diesem verknüpften Prozesse. Diese sind von nervösen Reizimpulsen, Hormonwirkungen und wechselnden funktionellen Zuständen von Organen abhängig.

Da die Schilddrüse und das Hormon derselben die Oxydationsprozesse und den Sauerstoffgrundverbrauch steigern, werden bei Hyperfunktion der Schilddrüse auch Kohlehydrate in vermehrter Menge abgebaut. Hierdurch beteiligt sich die Thyreoidea-funktion an der Blutzuckerregulation.

Eine Vorbedingung des gesteigerten oxydativen Zuckerabbaus ist eine vermehrte Glykosezufuhr zum Blut. Neben seiner die Verbrennung fördernden Wirkung mobilisiert daher das Thyroxin noch das Leberglykogen und senkt den Glykogengehalt der Leber. Ausserdem beschleunigt nach Lucke, Heydemann und Duensing das Thyroxin die Zuckerbildung aus Eiweiss, wodurch der Blutzuckerspiegel steigt, Körpereiwiss abgebaut wird, und die Stickstoffausscheidung zunimmt. Dies tritt bei hypophysektomierten Tieren deutlicher hervor.

Durch das thyreotrope Hypophysenvorderlappenhormon ist die Schilddrüse von der Funktion der Hypophyse abhängig, welche auf mannigfache Weise in die Blutzuckerregulation eingreift, wovon weiter unten in diesem Abschnitt noch eingehender die Rede sein wird.

## Die blutzuckerregulatorischen Systeme

Sämtliche Faktoren, welche den Blutzuckerspiegel beeinflussen, müssen sich bei einem konstanten Blutzuckerhalt im Gleichgewicht befinden. Insbesondere nach Nahrungszufuhr werden daher grosse Ansprüche an die Blutzuckerregulation gestellt.

Die blutzuckersteigernden und die blutzuckersenkenden Regulationskräfte müssen koordiniert sein und lassen sich in zwei grosse regulatorische Systeme einordnen:

1. Das *zerebral-hypophysäre Regulationssystem* oder *regulatorische System des Grundumsatzes*. Dieses sorgt für das Blutzuckergleichgewicht im Hunger. Seine Wirkung ist hauptsächlich eine blutzuckersteigernde, zum Ausgleich des basalen Zuckerverbrauchs.

2. Das *duodeno-pankreatische Regulationssystem* oder *alimentäre glykoregulatorische System*. Dasselbe tritt nach Nahrungszufuhr oder bei alimentären Reizimpulsen in Tätigkeit und wirkt in der Hauptsache blutzuckersenkend.

Eine Störung des Gleichgewichts zwischen diesen beiden Systemen ruft eine Änderung des Blutzuckergehalts hervor. Eine dauernde Störung in den regulatorischen Systemen kennzeichnet denjenigen Zustand, welcher seit alters als Diabetes mellitus bekannt ist. Der Angriffspunkt der Störung kann an jeder beliebigen Stelle des glykoregulatorischen Mechanismus liegen. Es kommt dabei zu dem klinischen Bilde des Diabetes, mit abnormen Blutzuckerwerten, Zuckerdiurese und evtl. Ketonurie.

*Der Diabetes ist somit keine einheitliche Erkrankung, sondern ein Symptomkomplex, bei dem Zuckerdiurese und Blutzuckersteigerung nur der Ausdruck einer Störung von Partialfunktionen der glykoregulatorischen Systeme sind.*

Den beiden Regulationssystemen entsprechend hat man mit zwei verschiedenen Diabetestypen zu rechnen. Der eine ist dadurch charakterisiert, dass sich die Stoffwechselstörung nur bei Nahrungsbelastung bemerkbar macht. Die Störung hat da hauptsächlich das alimentäre Regulationssystem getroffen.

Bei dem anderen Diabetestypus dagegen äussert sich die Stoffwechselstörung auch im Hunger. Es ist da eine Störung im glykoregulatorischen System des Grundumsatzes eingetreten.

Diese beiden Diabetestypen lassen sich klinisch bis zu einem gewissen Grade voneinander unterscheiden und werden im VIII. Abschnitt als Diabetes vom Typus A bzw. Typus B des näheren besprochen werden.

## Das zerebral-hypophysäre Regulationssystem

Die Regulation des Grundumsatzes ist eng mit der Tätigkeit des Nervensystems verknüpft. Sie findet durch ein intimes Zusammenwirken des Zentralnervensystems mit dem innersekretorischen System statt. Da die Hypophyse hierbei eine wichtige vermittelnde Rolle spielt, kann das glykoregulatorische System des Grundumsatzes als das *zerebral-hypophysäre* Regulationssystem bezeichnet werden.

## Zentralnervensystem und Zwischenstoffwechsel

Das Eingreifen des Nervensystems in den Zuckerumsatz ist seit dem Jahre 1857 bekannt, in welchem *Claude Bernard* den blutzuckersteigernden Effekt des Zuckerstichs entdeckte. Diese Hyperglykämie tritt nach einer Verletzung der Medulla oblongata auf, welche den Boden des vierten Ventrikels trifft. Später ist die Bedeutung des Zentralnervensystems für den Stoffwechsel immer eingehender erforscht worden, und man hat die regulatorisch wirksamen Zentren lokalisieren können.

## Blutzuckergehalt und Thermoregulation

Der normale Lebensprozess der höheren Organismen setzt eine Temperatur voraus, welche nur innerhalb enger Grenzen schwanken darf. Die erste Bedingung hierfür ist eine stetige Verfügung über den Energie liefernden Brennstoff im Organismus. Da dieser Brennstoff in erster Linie aus den Kohlehydraten im Blute besteht, bildet die Blutzuckerregulation ein wesentliches Moment bei der Thermoregulation des Organismus.

Bei den biologischen Oxydationsprozessen erfolgt nach *Ott* die Wärmebildung spontan in den Geweben. Durch eine Veränderung der Oxydationsintensität variiert die Wärmeproduktion. Die Körpertemperatur wird durch das Verhältnis zwischen Wärmeerzeugung und Wärmeabgabe bestimmt. Zur Beibehal-

tung einer konstanten Temperatur müssen sowohl Wärmebildung wie Wärmeabgabe geregelt werden. Die *Wärmebildung* hängt von der bei der Verbrennung umgesetzten Glykosemenge ab. Die *Wärmeabgabe* dagegen wird von der Wirkung vasomotorischer, respiratorischer und transpirationsregulatorischer Impulse seitens nervöser Zentren gesteuert, welche bei normaler Thermoregulation mit den stoffwechselregulierenden Zentren, die für die Wärmebildung zu sorgen haben, harmonisch koordiniert sein müssen. Wird dem Blute mehr Glykose zugeführt als in der Norm und die Verbrennungsintensität ausserdem gesteigert, so kommt es zu einer Temperatursteigerung, durch welche der Glykoseüberschuss aus dem Blute verschwindet. Die Körpertemperatur kann dabei steigen, der Blutzuckergehalt aber nach wie vor normal sein. Wenn der Glykoseüberschuss dagegen nicht verbrannt wird, steigt der Blutzuckergehalt, wobei Zucker im Urin ausgeschieden werden kann. Es entsteht da ein Diabetes. *Energetisch betrachtet wird daher ein Diabetes zum Äquivalent einer Temperatursteigerung und in gewissem Sinne gleichwertig mit einem Fieberzustand.*

Es ist also kein Zufall, dass temperaturregulierende und stoffwechselregulierende Zentren nahe beieinander in basalen Ganglien des Zentralnervensystems lokalisiert sind. Bei von Störungen in diesen Zentren verursachten Diabetesformen löst

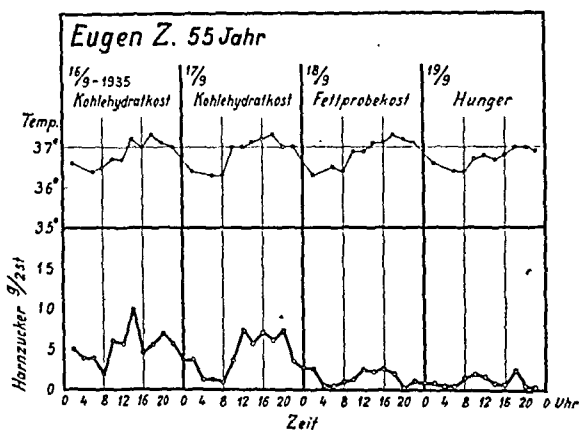


Abb. 39. Diabetes mit übereinstimmenden periodischen Variationen der Körpertemperatur (Rektalmessung) und Zuckerausscheidung (nach Möllerström).



eine hinzutretende Infektion mit Temperatursteigerung nicht selten eine starke Verschlimmerung der diabetischen Stoffwechsellaage aus. Beim Diabetes findet man bisweilen auch eine deutlich hervortretende Übereinstimmung zwischen Variationen der Körpertemperatur und der Zuckerausscheidung (s. Abb. 39).

## Einfluss der Gehirnzentren auf Stoffwechsel und Körpertemperatur

### *Die Rindenzentren des Grosshirns.*

*Eulenburg* und *Landois* hatten bereits 1876 gezeigt, dass eine schwache Reizung der motorischen Rindenzone eine Senkung der Temperatur der kontralateralen Extremität zur Folge hatte. Wurde die Rindenzone zerstört, so trat eine Temperatursteigerung ein, und die Differenz zwischen höchster und niedrigster Temperatur konnte bei diesen Versuchen bis 13° betragen.

*Medica* und *Andemino* haben bei Versuchen an Hunden nachgewiesen, dass eine Zerstörung der motorischen Rindenzone eine verringerte Ausscheidung von Stickstoff und Phosphor nach sich zieht. Eine Rindenreizung bewirkt dagegen eine gesteigerte Phosphorausscheidung. Dies deutet auf ein direktes Eingreifen in die chemischen Umsetzungsprozesse hin.

*Hitzig*, *Wood*, *Vulpian* u. a. fanden, dass auch andere Teile der Hirnrinde ausserhalb der motorischen Zone Temperaturveränderungen bewirken können.

Das Eingreifen der Hirnrinde in den Stoffwechsel ist wahrscheinlich sekundärer Art und erfolgt durch vasomotorische und trophische Störungen in der Muskulatur und den Geweben mit Veränderungen der chemischen Umsetzungsprozesse.

### *Subkortikale Zentren*

können direkt oder indirekt den chemischen Umsatz und die Wärmeregulation beeinflussen.

*Aronsohn* und *Sachs* stellten bei Reizung des Nucleus caudatus eine Erhöhung der Körpertemperatur sowie vermehrte Kohlensäureproduktion und Stickstoffausscheidung fest. *Aron-*

sohn fand, dass der Effekt der Reizung bei Kurarevergiftung ausblieb, aber wiederkehrte, sobald die Kurarewirkung verschwand. Er folgerte hieraus, dass die Temperatursteigerung durch erhöhten Stoffwechsel in der Muskulatur zustande kommt.

### *Thalamus.*

*Girard* (1886) konstatierte bei Reizung des Corpus striatum und Thalamus eine vorübergehende Temperatursteigerung mit Beschleunigung der Atmung und des Pulses. Gleichzeitig stiegen Sauerstoffverbrauch, Kohlensäureproduktion und Stickstoffausscheidung.

Nach *Zunz* und *La Barre* wird ausserdem die Insulinerzeugung in den Langerhansschen Inseln von Zentren im Thalamus auf dem Wege über den N. vagus beeinflusst.

*Vom Thalamus aus können zentripetale psychoreflektorische Impulse wahrscheinlich direkt in chemische Prozesse des Organismus eingreifen.*

### *Kleinhirn.*

Das Kleinhirn ist ein Reflexorgan für das motorische Nervensystem. Gewisse Teile des Kleinhirns, dem Corpus cerebelli entsprechend, üben u. a. einen tonisierenden Einfluss auf die Muskulatur aus. Die Bedeutung des Kleinhirns für den Stoffwechsel in der Muskulatur ist nicht näher bekannt. *V. Monakow* fand bei Kleinhirnaffektionen und experimentellen Kleinhirnverletzungen fettige Degeneration in der Muskulatur mit körnigem Zerfall der quergestreiften Muskelfasern. *Luciani* beobachtete bei Tierversuchen, dass sich manchmal ein Diabetes entwickeln konnte, wenn die Kleinhirnhemisphären entfernt worden waren. Es traten da Polyurie, Glykosurie und Ketonkörperausscheidung auf. Dieser Diabetes könnte jedoch möglicherweise auf einer Reizung benachbarter Teile der Medulla oblongata beruhen.

### *Hypothalamus.*

Die stoffwechselregulierenden Zentren im engeren Sinne sind im Hypothalamus in der Nähe der übrigen vegetativen Regulationszentren rings um den dritten Ventrikel lokalisiert.

*Dubois* entdeckte 1902 ein Zentrum in der Wand des dritten Ventrikels, ventralwärts unter dem *Aquaeductus Sylvii*, dessen Reizung bei den Versuchstieren einen Schlafzustand zur Folge hatte. Dabei änderte sich auch die Atmung zu einem dem *Cheyne-Stokesschen* ähnlichen Atmungstypus. Gleichzeitig nahm ferner die Glykogenbildung in der Leber ab.

*Aschner* (1912) fand, dass bei Verletzungen des Hypothalamus eine Störung des Kohlehydratstoffwechsels mit Glykosurie zustande kommen konnte.

*Leschke* (1920) beobachtete beim Diabetes Veränderungen im Zwischenhirn, namentlich an der Basis und den Seiten des dritten Ventrikels. *Camus, Gournay* und *Le Grand* (1923) konstatierten bei Versuchen an Kaninchen, dass eine Verletzung des Tuber cinereum eine Glykosurie und Diabetes verursachen konnte. Dieser entwickelte sich nur dann, wenn die Tuberkerne und der Nucleus paraventricularis von dem Trauma getroffen worden waren.

*Marinesco* und *Paulian* fanden bei Akromegalie mit Diabetes anatomische Veränderungen in den Kernen des Tuber cinereum und im Nucleus paraventricularis. Bei einem luetischen Diabetiker sahen *Marinesco* und *Nicolesco* doppelseitige Infiltrationen im Nucleus supraopticus und Nucleus paraventricularis.

*Morgan, Vonderahe* und *Malone* untersuchten die Kerne im Hypothalamus bei fünf Diabetikern und fünf nicht an Diabetes leidenden Personen. Bei sämtlichen Diabetesfällen fanden sie eine erhebliche Zellreduktion und einen Zellschwund, der 35—65 % der Norm entsprach. Namentlich die Ganglienzellen im Nucleus paraventricularis waren vermindert, aber es lagen auch Veränderungen im Nucleus tuberis und im zentralen Höhlengrau rings um den dritten Ventrikel vor.

*Himwich* und *Keller* haben eine Hyperglykämie bei direkter Reizung des Hypothalamus nachgewiesen. *D'Amour* und *Keller* fanden auch hypoglykämische Zustände bei Eingriffen am vorderen Hypothalamus.

*Strieck* hat bei Versuchen an Hunden eine kleine Menge Silbernitratlösung in den Hypothalamus eingespritzt und hierdurch eine Glykosurie ohne Veränderung von Temperatur, Puls

oder Atmung und mit normalem Grundumsatz hervorgerufen. Eine mässige Blutzuckersteigerung (160—260 mg%) und eine Zuckerdiurese mit 1,0—4,2 % Harnzucker blieben bis zum Tode des Tieres 2 Monate nach dem Eingriff bestehen. Dieser Diabetes, der von Durst, Polyurie und Abmagerung begleitet wurde, war insulinresistent, und der Nüchternblutzucker liess sich trotz Insulinbehandlung nicht auf normale Werte herabbringen. Bei der Autopsie wurde festgestellt, dass die Verätzung das zentrale Höhlengrau auf einer Seite getroffen hatte. Im Pankreas waren keine Inselveränderungen zu finden.

*Barris* und *Ingram* beobachteten bei Versuchen an Katzen eine vorübergehende Glykosurie und Hyperglykämie nach einer leichten Verletzung des Hypothalamus. In gewissen Fällen folgte auf diese Hyperglykämie eine dauernde Hypoglykämie. Dies war der Fall, wenn der vordere Hypothalamus geschädigt wurde, und in der Regel dann, wenn der Nucleus paraventricularis zerstört worden war. Die Tiere waren da auch abnorm insulinempfindlich. Bei Verletzungen anderer Gebiete des Hypothalamus stieg dagegen die Insulinempfindlichkeit nicht.

Eine schematische Darstellung der nervösen Ganglienhäufen im Hypothalamus nach *Fulton* mit der *Le Gros Clark*schen Nomenklatur auf der Grundlage der Arbeiten von *Rioch* und *Greving* wird in Abb. 40 S. 195 wiedergegeben.

## Die Koordination des Nervensystems mit dem hormonalen System

Zwischen den Ganglienzellen des Hypothalamus und der innersekretorischen Funktion der Hypophyse besteht ein enger Zusammenhang.

*Davis*, *Cleveland* und *Ingram* haben gefunden, dass eine doppelseitige Verletzung der lateralen Hypothalamuskern, an der Übergangsstelle zum Nucleus tuberis, die Tiere vor Pankreasdiabetes in derselben Weise schützt, wie die Entfernung der Hypophyse. *Berblinger* fasst daher Hypophyse und Hypothala-

lamus zu einer funktionellen neurohormonalen Einheit, der *Neurohypophyse*, zusammen. Dem im Hypothalamus befindlichen *Nucleus supraopticus* wird die Bedeutung eines nervösen Zentrums für die Hypophysenfunktion zugeschrieben.

Sowohl in basalen Zentren des Zwischenhirns als auch im Stiel und Mittellappen der Hypophyse hat *Scharrer* zwischen den Ganglienzellen eine Kolloidsubstanz gefunden, welche er für ein Sekretionsprodukt der Nervenzellen im Hypothalamus hielt. Diese Substanz liegt sowohl intrazellulär wie in angrenzenden Saftlücken. Sie wurde als ein Neurohormon aufgefasst, dessen Wirkung jedoch nicht mit Sicherheit bekannt ist. Gewisse Gründe sprechen dafür, dass es sich um ein Regulationshormon für den Wasserhaushalt handelt.

Bei Verletzung der Basis des Zwischenhirns konstatierte *Leschke* 1919, dass auch bei unversehrter Hypophyse ein Diabetes insipidus zustande kommen konnte. Auch *Hechst* hat einen Fall von Diabetes insipidus nach Encephalitis epidemica beschrieben, bei dem die Veränderungen in diesem Zwischenhirngebiet lokalisiert waren, mit völlig ungeschädigter Hypophyse.

*Greving* und *Pines* haben *nervöse Verbindungen* zwischen Hypophyse und Zwischenhirn nachgewiesen, welche einerseits vom *Nucleus supraopticus* und andererseits vom *Nucleus paraventricularis* ausgehen.

Durch den *Tractus hypophyseus supraopticus* verlaufen direkte Nervenverbindungen zwischen dem *Nervus opticus* und der Hypophyse. *Edinger* hat gezeigt, dass die Nervenverbindungen zwischen Hypophyse und *Nucleus supraopticus* nach Enukleation des Auges entarten. Es bestehen daher Möglichkeiten, dass *direkte Lichtimpulse auf dem Wege über den Nucleus supraopticus die Hypophysenfunktion beeinflussen und damit auf den gesamten chemischen Umsatz rückwirken können*.

*Jores* hat auch nachgewiesen, dass das Melanophorhormon im Mittellappen der Hypophyse bei Säugetieren deutliche quantitative Schwankungen bei wechselnden Lichtreizen aufweist. Während der Nacht enthält das menschliche Blut eine Substanz mit ähnlicher Wirkung wie die des Melanophorhormons.

*Jores* fand, dass diese Substanz, wie das Pigmenthormon, bei intrazerebraler Injektion eine Temperatursenkung und Blutzuckersteigerung bewirkt. Nach *Jores* können die optischen Impulse auf diesem Wege dem hormonalen System zugeführt werden. Der vegetative 24Stundenrhythmus, welcher sich u. a. in der periodischen Leberfunktion und in der endogenen Rhyth-

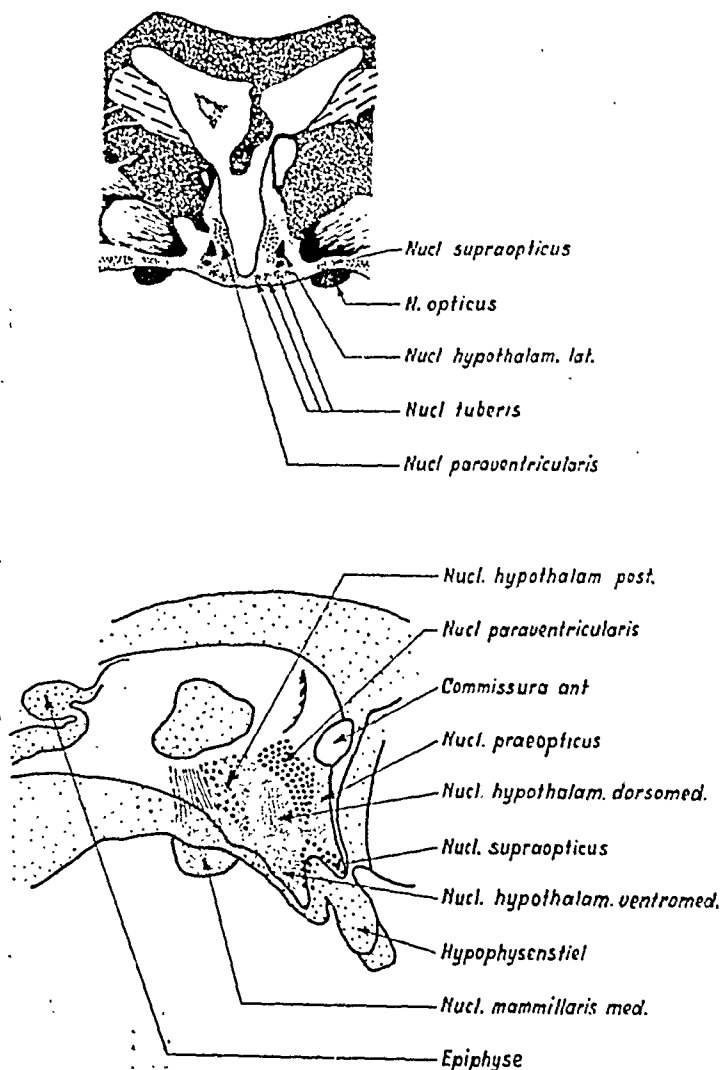


Abb. 40. Ganglienzentren im Hypothalamus (nach *Fulton*).

mik des Zwischenstoffwechsels bemerkbar macht, kann möglicherweise hierdurch von Lichtimpulsen auf dem Wege über den Nervus opticus — Nucleus supraopticus und die Hypophyse beeinflusst werden (vgl. S. 41).

*Hjalmar Holmgren* untersuchte den Leberhythmus bei dauernd im Dunkel gehaltenen Ratten und Meerschweinchen und kam zu dem Resultat, dass dem Licht als rhythmusauslösendem Faktor grosse Bedeutung zukommt, dass es aber ausserdem einen mit dem Licht koordinierten Faktor gibt, möglicherweise von hereditärer Art.

Nach Untersuchungen von *Popa* und *Fielding* existieren direkte *Venenverbindungen* zwischen Hypophyse und Hypothalamus. Eine Sekretion der Hypophyse kann dadurch die Ganglienzellen im Hypothalamus unmittelbar beeinflussen.

Nach *Popa* erstreckt sich ein Fortsatz des mit Ependym ausgekleideten dritten Ventrikels durch den Hypophysenstiel bis tief in die Hypophyse hinein und verzweigt sich dort fingerförmig; daher können aus der Hypophyse stammende Sekretionsprodukte direkt in die Zerebrospinalflüssigkeit im dritten Ventrikel hineingelangen und auch auf diesem Wege Einfluss auf zentrale Ganglien im Hypothalamus ausüben.

*Greving* und *Pines* haben direkte Nervenfasern nachgewiesen, welche durch das *Schütz'sche Bündel* in der Wand des dritten Ventrikels vom Nucleus paraventricularis zum dorsalen Vaguskern verlaufen. Von dort ziehen präganglionäre Fasern durch den Nervus vagus zum Pankreas und den Langerhans'schen Inseln. Die *Insulinproduktion* kann infolgedessen entweder durch primäre nervöse Reizimpulse vom Nucleus paraventricularis aus oder sekundär durch die sekretorische Hypophysenfunktion auf dem Wege über die zentralen Ganglien beeinflusst werden. Die Wirkung gewisser Hypophysenhormone wird wahrscheinlich auch vom Zentralnervensystem vermittelt.

Hypophyse und Zwischenhirn stehen also in einem wechselseitigen Abhängigkeitsverhältnis voneinander. Gewisse Hypophysenhormone scheinen die nervösen Zentren im Hypothalamus zu tonisieren, analog der Wirkung der Nebennieren und des Adrenalins auf die sympathischen Ganglien (s. S. 9).

Nach *Cushing* sollen die Sekretionsprodukte des hinteren Hypophysenlappens vor allem den Tonus des Vaguszentrums steigern.

Das Eingreifen des nervösen Reizimpulses in den chemischen Umsetzungsprozess wird durch die Wirkung spezifischer Substanzen vermittelt. Diese werden an den Endpunkten der Neurone gelegentlich der Nervenreizung frei gemacht. So wird nach *Dale, Feldberg* und *Brown* bei *Vagusreizung* Azetylcholin gebildet, welches den Reizungseffekt auslöst. Wenn die Nervenreizung aufhört, kommt die Azetylcholinbildung in den Vagusendigungen zum Stehen, wobei das bereits gebildete Azetylcholin von der in den Geweben vorhandenen Cholinesterase gespalten wird. Damit verschwindet der Reizungseffekt. Bei *Sympathikusreizung* entsteht nach *Cannon* in derselben Weise *Sympathin* an den Endigungen der Sympathikusneurone, eine Substanz mit adrenalinähnlicher Wirkung, deren chemische Natur aber noch unbekannt ist. Durch Vermittelung spezifischer Substanzen löst also die Nervenreizung die chemischen Reaktionsprozesse in Geweben und Organen aus, welche für ihren weiteren Verlauf von den Atmungsprozessen und der Kohlehydratverbrennung in den lebenden Zellen abhängig sind. Es ist möglich, dass die Nervenreizung auf diese Weise den Anstoß zu einem Elektronenaustausch gibt, welcher für die Funktionen des Atmungsferments beim biologischen Oxydationsprozess erforderlich ist (vgl. V. Abschnitt).

Das Nervensystem kann auf diese Art möglicherweise in die chemischen Vorgänge des Intermediärstoffwechsels eingreifen.

Die Insulinproduktion steht unter nervöser Kontrolle seitens des Vagus und Sympathicussystems, und nach *La Barre* enthält der rechte Vagus insulinsekretorische Nervenfasern. Abb. 41 zeigt nach *de Castro* die Innervation der Langerhansschen Inseln im Pankreas.

Eine Störung in der chemischen Umsetzung kann *entweder* bei veränderten Impulsen seitens nervöser Zentren im Zentralnervensystem und mit diesen koordinierter hormonenerzeugender Organe zustande kommen, *oder* bei einem Mangel an wirksamem Atmungsferment und Biokatalysatorsubstanzen, welche erforderlich sind, um das Eingreifen der Nervenimpulse in die



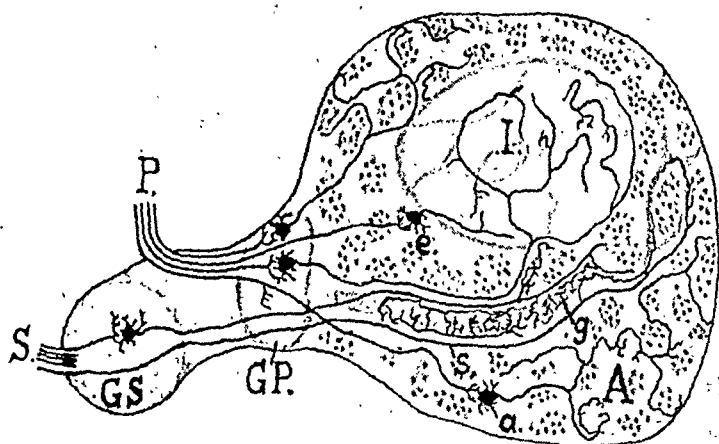


Abb. 41. Schematische Darstellung der Innervation der Pankreasazini (A) und der Langerhansschen Inseln (I) bei der Ratte. P Vagusfasern, S Splanchnicusfasern, GS Ganglien des Plexus solaris, Gp Ganglion extrapankreaticum, e juxtainsulare Ganglien, g vasomotorische, a sekretorische und s zentripetale (sensible) Nervenbahnen (nach de Castro).

chemischen Umsetzungsprozesse der lebenden Zellen zu vermitteln.

In Anbetracht dieser Verhältnisse kann man daher grundsätzlich einen von einer nervösen oder hormonalen Regulationsstörung verursachten *Regulationsdiabetes* und einen durch Mangel an vermittelnden Biokatalysatorsubstanzen endo- oder exogenen Ursprungs hervorgerufenen *Mangeldiabetes* unterscheiden. Es ist in der Regel nicht möglich, diese prinzipiellen Diabetesformen klinisch auseinanderzuhalten, da es sich oft um Kombinationsformen handelt und die Symptome übrigens oft gleichartig sind (vgl. S. 262).

## Die glandotropen Hypophysenhormone

Die innersekretorische Tätigkeit der Hypophyse besteht einmal aus der Bildung glandotroper Hormone, sodann aus der Produktion von Hormonen, welche direkt oder indirekt in den Stoffwechselprozess eingreifen. Die glandotropen Hormone sind als spezifische Wachstumshormone und Reizsubstanzen für die hormonerzeugenden Zellsysteme der endokrinen Drü-

sen aufzufassen. Sie haben Eiweisscharakter, ihr chemischer Bau ist aber sonst nicht näher bekannt.

Das Wechselspiel zwischen Inkretdrüsen, Hypophyse und Zwischenhirn ist für die Bildung derjenigen Arbeitshormone bestimmend, welche aktiv in die Kette der chemischen Umsetzungen eingreifen.

Durch Rückwirkung der endokrinen Drüsenhormone auf die glandotrope Hormonbildung der Hypophyse kommt durch die hemmenden oder anregenden Wirkungen der ersteren eine hormonale Autoregulation innerhalb des endokrinen Systems zustande.

Ein typischer Exponent für diese hormonale Autoregulation ist der periodische Menstruationszyklus, der durch das Zusammenwirken der gonadotropen Hypophysenvorderlappenhormone und des aktiven Sexualhormons der Ovarien entsteht.

Durch Arbeiten von *Zondek* und *Aschheim* (1926) sowie später von *Evans* (1934) wurde erwiesen, dass im Vorderlappen der Hypophyse zwei verschiedene gonadotrope Hormone gebildet werden, Prolan A und Prolan B, jenes das Follikelreifungshormon, dieses das Luteinisierungshormon, welches die Corpus-luteumbildung hervorruft. Das bei der Follikelreifung entstehende Follikelhormon, das *Follikulin*, dämpft die Bildung von Prolan A, stimuliert aber die Erzeugung von Prolan B. Das bei der Corpus-luteumbildung produzierte Corpus-luteumhormon, das *Lutein*, regt dagegen die Bildung von Prolan A an. Durch diese Wechselwirkung kommt ein periodisches Phänomen zustande, welches sich im Verlauf des Menstruationszyklus widerspiegelt.

Sexualhormone und gonadotrope Hypophysenhormone greifen wahrscheinlich in den Kohlehydratstoffwechsel ein. Der Glykogengehalt der Leber wechselt im Zusammenhang mit dem Brunstzyklus (S. 42). Die Wirkung der verschiedenen Hormone beim Stoffwechsel ist jedoch im einzelnen noch unbekannt.

### Pankreatotrope Hormone

*Anselmino*, *Herold* und *Hoffmann* (1933) fanden bei Tierversuchen, dass eine tägliche Behandlung mit einem wässrigen

Extrakt aus mit Azeton getrocknetem Hypophysenvorderlappen nach einiger Zeit eine Hypertrophie der Langerhansschen Inseln im Pankreas zur Folge hatte, mit Zellvergrößerung, gesteigerter Zellteilung und Neubildung von Inseln.

Markes und Young (1929) haben nachgewiesen, dass der Insulingehalt in diesen hypertrophischen Inseln zunahm. Young sowie auch Richardson haben ebenfalls gezeigt, dass eine kurzdauernde Behandlung mit Hypophysenvorderlappenextrakt eine Zunahme der Anzahl und Grösse der Langerhansschen Inseln bewirkt, dass aber eine länger dauernde Behandlung schwere degenerative Zelleränderungen in den Inseln verursacht. Dabei entwickelt sich bei den Versuchstieren ein dauernder Diabetes.

Durch spezielle Reinigung des Gesamtextrakts aus mit Azeton behandeltem Hypophysenvorderlappen mittels Ultrafiltration bei pH 5,4 konnten Anselmino und Hoffmann eine Fraktion abtrennen, welche eine vorübergehende Blutzuckersenkung mit dem Maximum 3—5 Stunden nach der Injektion hervorrief. Bei pankreaslosen Versuchstieren bewirkte der gereinigte Hypophysenextrakt keine Blutzuckersenkung, was auch von Antognetti und Scopinari bestätigt wurde. Der Extrakt, welcher auch die alimentäre Hyperglykämie und die Adrenalinhyperglykämie dämpft, scheint deshalb nur bei unversehrtem Pankreas zu wirken und muss daher eine pankreatotrop wirksame Substanz enthalten. Nach Collip soll diese Substanz die Langerhansschen Inseln zu gesteigerter Insulinbildung anregen. Es ist noch unsicher, ob dieser kurzdauernde Effekt eine Initialwirkung des Diabetes hervorrufenden Hypophysenhormons ist, oder ob die Hypophyse zwei verschiedene pankreatotrope Substanzen mit ungleicher Wirkung enthält.

Das pankreatotrope Hypophysenhormon wird bei Diabetikern nach Anselmino und Hoffmann im Urin ausgeschieden. Es kann aus diesem durch Alkoholfällung gewonnen werden.

Die blutzuckersenkende Wirkung des pankreatotropen Hormons ist verhältnismässig geringfügig und bleibt bei nicht genügend gereinigten Extrakten gänzlich aus. Dies beruht nach Zunz und La Barre auf dem Vorkommen einer anderen Substanz im Extrakt, welche die blutzuckersenkende Wirkung des

Insulins verhindert. Dieses *kontrainsulär wirkende Hormon* verursacht eine vermehrte Adrenalinbildung im Nebennierenmark und bewirkt eine Blutzuckersteigerung.

### Das kontrainsuläre Hypophysenvorderlappenhormon

*Lucke* beobachtete bei hypophysärer Insuffizienz das Auftreten klinischer Störungen der Kohlehydratregulation, mit niedrigen Nüchternblutzuckerwerten, gesteigerter alimentärer Hyperglykämie und erhöhter Nierenschwelle für die Zuckerausscheidung. Auch die Adrenalinhyperglykämie war stärker als normal. Auf die Blutzuckersteigerung folgte eine hypoglykämische Phase, welche so intensiv werden konnte, dass ein hypoglykämischer Schock eintrat. Es lag auch oft eine hochgradige Insulinüberempfindlichkeit mit Neigung zu spontanen Blutzuckersenkungen und hypoglykämischen Zuständen vor. *Lucke* führte diese klinischen Symptome bei hypophysärer Insuffizienz auf den Ausfall irgendeines Regulationsfaktors für den Kohlenhydratstoffwechsel zurück, der normalerweise in der Hypophyse vorhanden ist und der blutzuckersenkenden Wirkung des Insulins entgegenwirkt und dieselbe regelt. *Lucke* und seine Mitarbeiter konnten auch durch Tierversuche zeigen, dass sich aus dem Vorderlappen der Hypophyse ein Extrakt mit *blutzuckersteigernder* Wirkung gewinnen lässt. Die intramuskuläre oder intravenöse Zufuhr dieses Extrakts bewirkte bei Versuchen an Hunden eine mässige Blutzuckersteigerung mit einem Maximum nach 1 Stunde, welche nach 4—6 Stunden verschwunden war. Bei pankreasdiabelischen Tieren war die blutzuckersteigernde Wirkung des Extrakts viel stärker als bei normalen Tieren. Die Zuckerdiurese nahm gleichzeitig mit der Blutzuckersteigerung zu.

*Lucke, Heydemann und Hechler* (1933) haben noch nachgewiesen, dass der besagte Extrakt bei parenteraler Zufuhr die blutzuckersenkende Wirkung des Insulins unmittelbar aufhob. Dabei verschwand die Insulinüberempfindlichkeit bei den hypophysenlosen Versuchstieren. Bei peroraler Zufuhr war die Wirkung schwächer.

Im Vorderlappen der Hypophyse findet sich also ein *kontra-insulär* wirkendes Hormon. Nach *Lucke* ist das kontrainsuläre Hormon eine äusserst labile Substanz, welche in Wasser löslich, in Alkohol und Äther aber unlöslich ist. Dieselbe ist sehr temperaturempfindlich und büsst ihre Wirkung schon beim Eintrocknen im Vakuum bei niedriger Temperatur ein. Die Wirkung des Extrakts hört bereits während der Aufbewahrung bei der üblichen Temperatur nach einigen Monaten auf sowie bei kurzdauerndem Erwärmen, auch ohne Luftzutritt.

Das kontrainsuläre Hormon ist, wie mehrere andere Hypophysenhormone, kein Wachstumsfaktor für das Parenchym der endokrinen Drüsen und mithin nicht im eigentlichen Sinne glandotrop wirksam. Seine Wirkung setzt jedoch unversehrte Nebennieren mit ungeschädigten sympathischen Nervenverbindungen voraus. Der Effekt des kontrainsulären Hormons hört auf, wenn die Nebennieren entfernt oder die Nerven derselben verletzt werden.

Bei intraspinaler Zufuhr direkt in den Liquor cerebrospinalis bekommt das kontrainsuläre Hormon eine beträchtlich stärkere blutzuckersteigernde Wirkung als bei intravenöser oder intramuskulärer Verabreichung. Nach *Lucke* entleert sich das Hormon aus dem Vorderlappen der Hypophyse durch den Ductus hypophyseus direkt in den dritten Ventrikel. Von dort aus beeinflusst es unmittelbar die Sympathicuszentren im Hypothalamus und löst daher dann durch das sympathische Nervensystem eine gesteigerte Adrenalinbildung im Nebennierenmark aus. Hierdurch kommt schliesslich der blutzuckersteigernde Effekt des kontrainsulären Hormons zustande. *Diese Hormonwirkung wird also vom sympathischen Nervensystem vermittelt.*

*Lucke* und *Werner* (1938) haben bewiesen, dass die Bildung des kontrainsulären Hormons von dem sinkenden Blutzuckergehalt und nicht von einer direkten Insulinwirkung ausgelöst wird. Wird beim Versuchstier bei der Insulinzufuhr die Blutzuckersenkung durch gleichzeitige Verabreichung von Glykose oder Adrenalin verhindert, so lässt sich das kontrainsuläre Hormon im Liquor cerebrospinalis nicht nachweisen. Wenn dagegen die Blutzuckersenkung nicht verhindert wird, tritt das kon-



tergeleitet zu werden. Durch vermittelnde Vaguszentren wird im Vorderlappen der Hypophyse eine Hormonbildung ausgelöst, welche durch den Liquor cerebrospinalis sympathische Zentren beeinflusst. Von diesen gehen Impulse auf dem Wege über den Sympathicus und die Nn. splanchnici zum Nebennierenmark. Dort wird eine Adrenalinbildung ausgelöst, welche schliesslich die Blutzuckersteigerung bewirkt, die den blutzuckersenkenden Effekt des Insulins kompensiert.

In dieser neurohormonalen Reflexbahn gibt es viele Möglichkeiten für Störungen, welche sämtlich eine Verschiebung der normalen Blutzuckerregulation mit einem gesenkten oder erhöhten Blutzuckergehalt bewirken, abhängig von dem Punkt, an welchem die Schädigung einsetzt. Dabei kann ein Diabetes vom Regulationstypus oder *Regulationsdiabetes* entstehen.

### Das corticotrope Hypophysenvorderlappenhormon

*Smith* (1927) zeigte bei Versuchen an Ratten, dass die Nebennierenrinde nach Hypophysektomie atrophisch wird. Nach Implantation von Hypophysenvorderlappengewebe konnte bei den Versuchstieren diese Rindenatrophie wieder verschwinden. *Evans, Meyer und Simpson* (1932) fanden, dass auch ein wässriger Extrakt aus Hypophysenvorderlappen der Atrophie der Nebennierenrinde bei den hypophysenlosen Tieren entgegenwirkte.

*Anselmino, Hoffmann und Herold* (1933) sowie unabhängig von diesen *Collip, Anderson und Thompson* (1933) stellten ein von den übrigen Hypophysenvorderlappenhormonen verschiedenes corticotropes Hormon dar, welches Wachstum und Hypertrophie der Nebennierenrinde, namentlich der Zona fasciculata derselben, bewirkte. Dasselbe ruft eine gesteigerte Cortinbildung in der Nebennierenrinde hervor.

Das Cortin, das von *Kendall* aus der Nebennierenrinde dargestellte kristallinische Hormon, bringt nach *Russel und Craig* (1938) den Glykogengehalt der Leber und auch den Blutzuckerspiegel zum Steigen, übt aber keinen Einfluss auf den Glykogenbestand der Muskulatur aus. *Long und Katzin* (1938) fan-

den nach Cortinbehandlung subnormale Muskelglykogenwerte bei hypophysenlosen Tieren, auch bei sehr hohem Leberglykogengehalt.

Die Mitwirkung des Cortins ist wahrscheinlich für den Phosphorylierungsprozess von Bedeutung (vgl. IV. Abschnitt). Dasselbe wirkt dadurch in hohem Grade auf die Umsetzung der Kohlehydrate und Fette sowie auch auf die Resorption derselben ein (S. 79).

Durch das corticotrope Hormon kann mithin die Hypophyse stark in den Kohlehydrat- und Fettstoffwechsel eingreifen.

### Das thyreotrope Hypophysenvorderlappenhormon

*Adler* (1914) hat festgestellt, dass nach Hypophysektomie eine Atrophie der Schilddrüse mit verminderter Thyroxinbildung eintritt. *Smith* (1916) zeigte, dass diese Schilddrüsenatrophie nach Implantation von Hypophysenvorderlappengewebe zurückging. *Loeb* (1929) konnte auch die atrophische Schilddrüse nach Behandlung mit einem wässrigen Extrakt aus Hypophysenvorderlappen wieder zum Wachsen bringen. Die Hypophyse muss daher eine Substanz enthalten, welche die Schilddrüse und die Funktion derselben beeinflusst.

Das *thyreotrope Hormon* ist nach *Junkmann* und *Schöller* ein nicht ultrafiltrabler, niedermolekularer Eiweisskörper von Albumose- oder Peptoncharakter, welcher sehr thermolabil ist und schon bei kurzem Erwärmen auf 60° zerstört wird. In wässriger Lösung verschwindet die Wirkung binnen kurzer Zeit.

Das thyreotrope Hormon bewirkt ein Wachstum des Schilddrüsengewebes und eine Vermehrung des Follikelepithels mit gesteigerter Mitosenbildung sowie eine Verminderung des Kolloidgehalts in den Zysten. Die Thyroxinbildung nimmt hierbei zu.

Nach *Eitel* und *Loeser* (1932) ruft das thyreotrope Hormon ebenso wie Thyroxinbehandlung eine Verminderung des Glykogengehalts der Leber hervor. Diese tritt nach 3—4tägiger Behandlung ein. Bei schilddrüsenlosen Tieren hat das thyreotrope Hormon nicht diese Wirkung.



Die Abnahme des Leberglykogens unter dem Einfluss des thyreotropen Hormons geht mit einer Steigerung des Sauerstoffverbrauchs und einer Erhöhung des Grundumsatzes einher. Diese stellen sich nach und nach ein und nehmen in dem Masse zu, wie die Schilddrüsenfunktion durch die Wirkung des thyreotropen Hormons gesteigert wird.

*Di Benedetto* (1932) hat bei Tierversuchen nachgewiesen, dass die Insulinempfindlichkeit in hohem Grade steigt, wenn sowohl die Schilddrüse als auch die Hypophyse entfernt wird. Dabei sinkt der Blutzuckergehalt auf hypoglykämische Werte. *Houssay* und ebenso *Cope* und *Marks* (1934) haben gefunden, dass die Insulinempfindlichkeit bei den in dieser Weise behandelten Tieren nach Verabreichung von Hypophysenextrakt zurückgeht. Perorale Zufuhr von Schilddrüsenpräparaten scheint den Blutzuckerspiegel nicht in nennenswertem Grade zu beeinflussen, aber *Lucke*, *Heydemann* und *Duensing* stellten fest, dass intravenöse Thyroxinjektionen bei hypophysenlosen Tieren eine stärkere Blutzuckersteigerung bewirken als bei normalen Tieren. Gleichzeitig nimmt die Stickstoffausscheidung zu.

Nach *Soskin*, *Levine* und *Heller* (1939) entwickelt sich nach Hypophysektomie durch Ausfall des thyreotropen Hypophysenvorderlappenhormons eine Atrophie des Schilddrüsenorgans. Die Folge davon ist eine ungenügende Thyroxinbildung. Das Thyroxin ist wirksam beim Übergang des Eiweisses in Kohlehydrate. Darum nimmt bei Mangel an thyreotropem Hormon die Zuckerbildung aus Eiweiss ab, wodurch der Blutzuckerspiegel sinkt. Wird Thyroxin zugeführt, so steigt die endogene Zuckerbildung aus Eiweiss, wobei der Blutzuckergehalt wieder zunimmt.

Thyroxinzufuhr setzt dagegen die Insulinüberempfindlichkeit der hypophysenlosen Tiere nicht herab. Dies hängt wahrscheinlich mit der Eigenschaft des Thyroxins, Leberglykogen zu mobilisieren, zusammen, wodurch die Glykogenspeicherung in der Leber gehindert wird. Durch den Glykogenmangel in der Leber fehlt das Material zur Blutzuckerbildung, welches notwendig ist, um der Insulinhypoglykämie regulatorisch zu begegnen. Dadurch tritt eine Insulinüberempfindlichkeit ein, die von der Wirkung des Thyroxins nicht beeinflusst wird.

## Stoffwechselregelnde Hypophysenvorderlappen- hormone

Das *kontrainsuläre* Hormon ist ein rein regulatorisches Hypophysenhormon, welches in der oben angegebenen Weise auf dem Wege über das Nebennierenmark wirkt. Es greift nicht direkt in den Stoffwechselprozess ein.

Das *thyreotrope* Hormon ist auch ein stoffwechselregelndes Hormon in dem Sinne, dass es durch die Mitwirkung der Schilddrüse den Eiweissumsatz und den Übergang des Eiweisses in Zucker beherrscht. Es ist jedoch eigentlich zu den glandotropen Hormonen zu rechnen.

Neben diesen Hormonen gibt es im Vorderlappen der Hypophyse mehrere andere bekannte solche, welche einen regulierenden Einfluss auf den Stoffwechsel ausüben und bei der Blutzuckerregulation mitwirken. Mehrere von ihnen sind heutzutage erst unvollständig bekannt, und der Mechanismus ihrer Wirkung ist nicht klar. Sie sind jedoch in Betracht zu ziehen, wenn man sich ein vollständigeres Bild von der Bedeutung der Hypophyse für den Kohlehydrathaushalt und vom Eingreifen derselben in die Blutzuckerregulation machen will. Da es sich zum Teil hierbei um Ergebnisse der Hypophysenforschung aus den allerletzten Jahren handelt, bei welchen die Resultate der einzelnen Forscher nicht selten einander widersprechen, herrscht noch Unsicherheit in bezug auf die endgültige Klärung der einschlägigen Probleme.

### Der grundumsatzsteigernde Faktor

Houssay (1933) und Péter (1934) wiesen im Vorderlappen der Hypophyse einen grundumsatzsteigernden Faktor nach, welcher von der Schilddrüse unabhängig war und viel rascher wirkte als das thyreotrope Hormon. Schon 1—5 Stunden nach der Injektion stieg der Grundumsatz und nahm nach 48 Stunden wieder ab.

Nach O'Donovan und Collip (1938) ist dieser grundumsatzsteigernde Faktor im Gegensatz zum thyreotropen Hormon und

den übrigen Hypophysenvorderlappenhormonen sehr hitzebeständig und alkaliresistent. Möglicherweise kann derselbe nach *Collip* mit den Melanophorhormon aus der Pars intermedia der Hypophyse identisch sein, welches eine ähnliche Wirkung hat.

Der gesteigerte Grundumsatz wird in diesem Falle nicht von einer vermehrten Stickstoffausscheidung begleitet und geht nicht mit einer Blutzuckererhöhung Hand in Hand. Ebenso wenig gibt es nach *Neufeld* und *Collip* (1939) irgendwelche Anhaltspunkte für eine Zunahme der Kohlehydratverbrennung. Da der vermehrte Sauerstoffverbrauch mit einem herabgesetzten respiratorischen Quotienten einhergeht, muss der grundumsatzsteigernde Faktor entweder eine erhöhte Fettverbrennung bewirken oder eine Umwandlung von Fett in Kohlehydrate verursachen. Womöglich ist die Wirkung des grundumsatzsteigernden Faktors eine Partialfunktion des fettumsatzregulierenden Hypophysenhormons.

### Der glykotrope Faktor

*Cope* und *Marks* (1934) fanden, dass tägliche Injektionen eines Hypophysenvorderlappenextrakts Kaninchen insulinresistent machten. *Young* (1936) konnte durch Vorbehandlung der Versuchstiere mit Hypophysenextrakt den blutzuckersenkenden Effekt des Insulins vollständig unterdrücken. Nach *Himsworth* und *Scott* (1938) ist eine reichliche Glykosezufuhr die Voraussetzung für die Entstehung dieser Insulinresistenz.

Im Gegensatz zur kontrainsulären Wirkung, welche rasch vorübergeht und von einer vermehrten Adrenalinbildung mit Blutzuckersteigerung verursacht wird, handelt es sich um ein langsames Ansteigen der Insulinresistenz, welche erst nach 2—3 Tagen manifest wird. Dabei tritt keine direkt blutzuckersteigernde Wirkung ein.

*Marks* (1936) hat gezeigt, dass Versuchstiere, welche auf diese Weise insulinresistent geworden waren, einen gesteigerten Leberglykogengehalt hatten; die Muskulatur war aber ärmer an Glykogen als bei den unbehandelten Kontrolltieren.

Auch bei behandelten Versuchstieren, welche auf Insulininjektionen nach wie vor mit einer normalen Blutzuckersenkung reagierten, war die Muskulatur glykogenärmer als bei den unbehandelten Tieren. Der Blutzucker muss daher während der Insulinwirkung bei diesen mit Hypophysenvorderlappenextrakt behandelten Tieren entweder auf irgendeine andere Weise als durch Speicherung als Muskelglykogen verschwinden, oder das Leberglykogen wird nicht normal mobilisiert, sondern bleibt in der Leber zurück.

Nach *Mark und Young* findet sich im Vorderlappen der Hypophyse ein *glykotroper Faktor*, welcher das Glykogen in der Leber zurückhält und die Glykogenbildung in der Muskulatur während der Wirkung des Insulins verhindert. Bei längere Zeit dauernder Behandlung setzt nach *Young* (1938) der glykotope Faktor die Kohlehydrattoleranz der Tiere herab und löst dadurch einen Diabetes aus.

Die Wirkung des glykotropen Faktors auf das Leberglykogen ist nach *Himsworth und Scott* sowohl von der Schilddrüse wie von der Hypophyse und Nebenniere unabhängig. Wahrscheinlich greift derselbe direkt in die Glykogenproduktion der Leber ein.

Die Wirkung des glykotropen Faktors im einzelnen sowie die Beziehung desselben zum corticotropen Hormon, welches ebenfalls durch Vermittlung der Nebennieren den Glykogengehalt der Leber zum Steigen bringt, ist noch nicht endgültig geklärt.

### Der glykostatische Faktor

*Russel* hat 1936 gezeigt, dass hypophysenlose Ratten gänzlich normale Blutzucker-, Leber- und Muskelglykogenwerte haben konnten, solange sie reichlich Nahrung erhielten. Im Hunger verringerte sich dagegen der Kohlehydratvorrat der Tiere rascher als bei normalen Tieren mit unversehrter Hypophyse (vgl. S. 41).

*Russel und Bennet* fanden, dass die Zufuhr eines speziell dargestellten Hypophysenvorderlappenextrakts diese abnorme Gly-

kogenverminderung bei den hungernden hypophysenlosen Tieren verhindern konnte. Dabei war die Beeinflussung des Muskelglykogens eine erheblich stärkere als die Wirkung auf Leberglykogen und Blutzuckergehalt, welche einander parallel gingen.

*Russel* und *Bennet* nahmen das Vorkommen eines *glykostatischen Faktors* im Vorderlappen der Hypophyse an, welcher das Glykogen in der Muskulatur zurückhält, aber Leberglykogen und Blutzuckerspiegel weniger beeinflusst.

Der glykostatische Faktor wirkt unabhängig von den Nebennieren und nur bei relativ kurzdauernder Behandlung. Bei längerer Behandlungsdauer, 2—3 Wochen, hat *Bennet* konstatiert, dass die Versuchstiere refraktär wurden, und dass der glykostatische Faktor bei ihnen nicht länger wirksam war.

Nach *Black* (1935) findet man bei dem ketogenen Fettstoffwechselhormon der Hypophyse (S. 217) bei länger dauernder Behandlung ein ähnliches Refraktärstadium. Daher ist es nach *Russel* wahrscheinlich, dass die Wirkung des glykostatischen Faktors eine Teilfunktion des Effekts des ketogenen Fettstoffwechselhormons ist. Diese Frage ist jedoch nicht endgültig geklärt. Ebenso wenig ist der Mechanismus im einzelnen bei der Wirkung des glykostatischen Faktors des näheren bekannt.

### Das kohlehydratstoffwechselregelnde Hormon

*Anselmino* und *Hoffmann* (1934) fanden im Vorderlappen der Hypophyse eine Substanz, welche auf den Glykogengehalt der Leber stark senkend wirkte. Eine Substanz mit ähnlicher Wirkung und entsprechenden Eigenschaften fanden sie auch im Blutserum und Urin von Diabetikern, im normalen Urin sowie im Blutserum gesunder Personen nach starker Kohlehydratbelastung.

Bei Versuchen an hypophysenlosen Hunden trat die das Leberglykogen herabsetzende Substanz im Blutserum nach Kohlehydratbelastung nicht auf. Die wirksame Substanz im Blut und Urin muss daher hypophysären Ursprungs sein.

Dieses Hypophysenhormon unterscheidet sich von den mei-

sten anderen Hypophysenvorderlappenhormonen durch seine Wärmeresistenz; es wird beim Erwärmen auf 60° nicht beeinflusst und weist nur eine mässige Empfindlichkeit gegen Kochen auf. Es ist in Alkohol, Azeton und Äther unlöslich und wird von sowohl Alkalien wie Säuren zerstört. In wässriger Lösung bei pH 5,3 ist es ultrafiltrabel, aber nicht bei pH 9,4.

Das kohlehydratstoffwechselregelnde Hormon bewirkt eine *Senkung des Glykogengehalts der Leber*, welche mehrere Stunden anhält, mit einem Minimum 2—4 Stunden nach der Injektion. Der Glykogengehalt kann dabei nach 2 Stunden bei hinreichender Dosis auf  $\frac{1}{10}$  des Ausgangswerts sinken.

Unter dem Einfluss des kohlehydratstoffwechselregelnden Hormons sinkt auch der Gehalt der Leber an ungesättigten Fettsäuren. Die maximale Fettsäuresenkung fällt jedoch nach *Anselmino, Effkemann und Hoffmann* nicht mit der Glykogenverminderung zusammen, sondern tritt erst 6—8 Stunden nach der Injektion ein. Am Zeitpunkt der stärksten Fettsäureverarmung ist der Glykogengehalt der Leber bereits auf normale Werte gestiegen.

Das kohlehydratstoffwechselregelnde Hypophysenhormon tritt im Blutserum nach einseitiger Kohlehydratbelastung auf. Wird gleichzeitig Fett zugeführt, oder bei gewöhnlicher gemischter Kost, so scheint die Hypophyse kein Kohlehydratstoffwechselhormon an das Blut abzugeben. *Anselmino und Hoffmann* (1936) haben auch nachgewiesen, dass grosse Insulindosen das Auftreten des Hypophysenhormons im Blutserum bei hypoglykämischen Zuständen verhindern. Kleine Insulindosen hingegen haben keinen Einfluss auf das Erscheinen des leberglykogensenkenden Hormons nach Kohlehydratbelastung. Der Blutzuckerspiegel wird von dem kohlehydratstoffwechselregelnden Hypophysenhormon nicht in entsprechendem Grade beeinflusst wie das Leberglykogen.

*Anselmino* (1937) und *Merten* (1939) fanden bei Versuchen an Ratten keine Wirkung auf den Blutzuckergehalt trotz Leberglykogensenkung unter dem Einfluss des Kohlehydratstoffwechselhormons. *Merten und Hinsberg* (1939) konnten indes bei intravenöser Injektion von gereinigtem Ultrafiltrat (pH 5,4) bei Kaninchen und Hunden eine deutliche Blutzuckerstei-

gerung mit einem Maximum schon nach 15—30 Minuten erzielen, welche entweder rasch verschwand, oder mehrere Stunden bestand, um dann allmählich auf normale Werte abzusinken.

Merten (1939) konstatierte einen insulinantagonistischen Effekt des kohlehydratstoffwechselregelnden Hormons, ähnlich dem von dem glykotropen Faktor ausgelöst. Beachtenswert ist dabei, dass im ersteren Falle eine Senkung des Glykogengehalts der Leber erfolgt, im letzteren dagegen eine Zunahme. Es war jedoch auch bei langdauernder Behandlung mit grossen Mengen Ultrafiltrat (pH 5,3), welches das kohlehydratstoffwechselregelnde Hormon enthält, nicht möglich, eine dauernde Störung des Kohlehydratstoffwechsels oder einen Dauerdiabetes zu erzielen, da die Tiere schon nach kurzer Behandlungsdauer gegen die Wirkung des kohlehydratstoffwechselregelnden Hypophysenhormons resistent wurden.

Unter dem Einfluss des Kohlehydratstoffwechselhormons steigt der Sauerstoffverbrauch, und der Gesamtumsatz wird grösser. Dabei nehmen auch Stickstoffausscheidung und Reststickstoff im Blute zu, wahrscheinlich durch gesteigerte Umsetzung von Eiweiss mit Bildung von Zucker aus demselben. Hierbei wirkt die Schilddrüse nicht mit. Dagegen ist es möglich, dass das kohlehydratstoffwechselregelnde Hypophysenhormon ein thymotropes Hormon ist, dessen Wirkung von der Thymusfunktion abhängig ist.

### Das thymotrope Hormon und Thymushormon

Durch Untersuchungen von Walhypophysen (1939—41) sind Bomskow und seine Mitarbeiter zu der Anschauung gelangt, dass das kohlehydratstoffwechselregelnde Hypophysenhormon eine *thymotrope* Wirkung hat und die Bildung eines *Thymushormons* auslöst.

Das Thymushormon ist nach Bomskow ein Wachstumshormon, welches bei Hyperfunktion eine Akromegalie oder bei gleichzeitigem Insulinmangel einen Diabetes hervorrufen kann.

Auch das normale Wachstum ist an eine gesteigerte Thymusfunktion gebunden.

Nach *Bomskow* ist eine unversehrte Thymusdrüse eine Voraussetzung für die Wirkung des thymotropen Hypophysenhormons. *Bomskow* hat bei Versuchen an Ratten gefunden, dass die Wirkung des thymotropen Hormons verschwindet, wenn die Thymusdrüse durch Röntgenbestrahlung geschädigt wird.

Das thymotrope Hypophysenhormon hat wie die übrigen Vorderlappenhormone Eiweisscharakter, aber das eigentliche Thymushormon ist nach *Bomskow* ein Sterin, welches in der Lipoidfraktion der Thymussubstanz vorhanden ist.

Das Thymushormon ist nach *Bomskow* in seiner Wirkung sehr aktiv, und verlässt normalerweise die Thymusdrüse in Lymphozyten gebunden. Das Thymushormon ruft auch eine Lymphozytose hervor. In hinreichender Menge senkt es bei parenteraler Zufuhr den Leberglykogengehalt binnen 6 Stunden auf einen Bruchteil normaler Werte. Das Hormon übt einen starken Einfluss auf den Glykogengehalt der Herzmuskulatur aus und verursacht bei intensiverer Wirkung Herzschwäche. Der Blutzuckerspiegel steigt unter der Einwirkung des Thymushormons, wobei eine Zucker- und Ketonkörperausscheidung eintreten kann.

Nach *Bomskow* hemmt das Thymushormon die Entwicklung der Geschlechtsdrüsen, obwohl es sonst ein Wachstum auslöst.

Das thymotrope Hypophysenhormon übt keinen Einfluss auf die Schilddrüse aus. Dagegen scheint das Thymushormon die Bildung des thyreotropen Hypophysenhormons zu beeinflussen und dadurch indirekt auf die Schilddrüsenfunktion einzuwirken. Es ruft eine Kolloidansammlung in der Schilddrüse hervor, lässt aber den Grundumsatz unberührt.

Obgleich Thyroxin und Thymushormon beide Leberglykogen mobilisieren, ist es nur das Schilddrüsenhormon, welches den Grundumsatz steigert. Es liegt daher wahrscheinlich ein prinzipieller Unterschied im Kohlehydratstoffwechsel unter dem Einfluss von Schilddrüsenhormon und Thymushormon vor. Im ersteren Falle wird der Grundumsatz gesteigert, und der Kohlehydratabbau erfolgt unter Freimachung von Energie. Im letzteren hingegen ist der Grundumsatz unverändert, und



die Kohlehydrate werden beim Zell- und Gewebswachstum verbraucht. Es ist möglich, dass der biologische Kohlehydratabbau in den beiden Fällen verschieden verläuft, entweder als eine gewöhnliche, energiebildende Hexo-Triosenspaltung oder als ein Hexo-Pentosenabbau. Hierüber ist jedoch noch nichts bekannt.

Die Frage, ob das kohlehydratstoffwechselregelnde Hypophysenhormon nach *Anselmino* und *Hoffmann* mit *Bomskows* thyrotropem Hormon identisch ist, muss bis auf weiteres offenbleiben. *Anselmino* und *Lotz* (1941) haben die Beobachtungen von *Bomskow* nicht bestätigen können, aber da sie mit verschiedenen Präparaten gearbeitet haben, der eine mit Extrakt aus Walhypophyse, wobei die Substanz in grösserer Menge gewonnen wird, die anderen mit dem fabrikmässig hergestellten Präparat Präphyson, kann dies womöglich die Ursache der verschiedenen Resultate sein. Das Thymushormon ist daher noch unsicher (vgl. S. 47).

### Das fettstoffwechselregelnde Hormon

Das Eingreifen der Hypophyse in den Fettstoffwechsel ist schon 1925 nachgewiesen worden. In diesem Jahre erhielt *Raab* aus Hypophysenvorderlappen einen Extrakt, welcher bei Einspritzung ein Sinken des Fettgehalts im Blute bewirkte. Die wirksame Substanz wurde von *Raab* als *Lipoitrin* bezeichnet. Unter dem Einfluss des Lipoitrins nahm auch der Fettgehalt der Leber zu.

Beim Pankreasdiabetes tritt eine starke Ketonurie auf. *Housay* (1929) fand, dass diese nach Hypophysenexstirpation nachlässt oder verschwindet.

*Burn* und *Ling* (1930) beobachteten bei Versuchen an Ratten, dass die Tiere mit einer Ketonkörperausscheidung reagierten, wenn sie fettreiches Futter erhielten und mit einem alkalischen Hypophysenextrakt behandelt wurden. Dabei war die Ketonkörperbildung bei Weibchen stärker als bei Männchen. *Anselmino* und *Hoffmann* (1931) stellten aus dem Vorderlappen der Hypophyse einen Extrakt dar, der bei Injektion

nach einigen Stunden eine vermehrte Ketonkörperbildung und Ketonurie bewirkte. Die wirksame Substanz ist in Wasser löslich, wird von Alkohol ausgefällt, und ist in schwach alkalischer Lösung bei pH 8,5 ultrafiltrabel. Sie ist wärmeempfindlich und verliert ihre Wirkung schon nach 15 Minuten langer Erwärmung auf 60°. Der isoelektrische Flockungspunkt derselben entspricht pH 5,4.

Ausser im Vorderlappen der Hypophyse lässt sich dieses Hormon noch im Urin nachweisen, namentlich im Urin gewisser Diabetiker und Schwangerer, im Diabetikerblut sowie im Blutserum Gesunder nach Nahrungsbelastung mit Fett. Die ketogene Substanz tritt bei normalen Personen schon 4 Stunden nach der Fettbelastung auf. Werden gleichzeitig mit dem Fett auch Kohlehydrate gegeben, so unterbleibt die Bildung des ketogenen Hormons, und dasselbe kann da im Blut nicht nachgewiesen werden. Es ist daher ein *Fettstoffwechselhormon* und tritt auch im Hunger auf. Wird da Glykose verabreicht, so verschwindet normalerweise die ketogene Wirkung des Blutes. Bei der üblichen gemischten Kost lässt sich in der Norm keine ketogene Wirkung des Blutserums nachweisen.

Das ketogene Hormon bewirkt schon 2 Stunden nach subkutaner Injektion eine Ketonämie und Ketonkörperausscheidung, welche nach weiteren 3—4 Stunden verschwindet. Dabei steigt hauptsächlich die  $\beta$ -Oxybuttersäure, so dass das normale Verhältnis  $\beta$ -Oxybuttersäure : Gesamtazeton = 1—3 : 1 unter dem Einfluss des ketogenen Hormons zu dem Verhältnis 7—10 : 1 verschoben wird. Unter der Einwirkung des Hormons steigt nach *Effkemann* der Gehalt der Leber an ungesättigten Fettsäuren und erreicht bereits 45—90 Minuten nach der Injektion ein Maximum. Dabei sinkt der Glykogengehalt der Leber. Im Gegensatz hierzu steigt nach *Steppuhn* der Muskelglykogengehalt, aber der Blutzuckerspiegel bleibt unverändert. Werden die Nn. splanchnici durchtrennt, so verschwindet der Einfluss auf das Muskelglykogen.

*Campbell* (1938) fand bei Versuchen an Ratten im Gegensatz zu *Effkemann*, dass während der Wirkung des ketogenen Hormons der Glykogengehalt der Leber parallel dem Fettgehalt stieg. Die widersprechenden Befunde können vielleicht auf die

rhythmische Lebertätigkeit mit spontanen Variationen in der Arbeitsleistung des Organs zurückzuführen sein.

*Best* und *Campbell* (1936) haben festgestellt, dass der Fettgehalt der Leber nach 3tägiger Behandlung mit Fettstoffwechselhormon um über 300 % steigen konnte, bis auf 13,4 % des Lebergewichts. Dabei nahm das Depotfett des Organismus im übrigen ab. Gleichzeitig mit der Vermehrung des Leberfetts steigerten sich die Ketonurie und der Ketonkörpergehalt des Blutes.

*Munoz* beobachtete eine Zunahme der Fettsäuren, des Cholesterins und der Phosphatide im Blut nach Behandlung mit Hypophysenvorderlappenextrakt, und *Steppuhn* fand, dass die maximale Steigerung dabei schon binnen 2 Stunden nach der Injektion eintrat.

Unter dem Einfluss des ketogenen Fettstoffwechselhormons scheint somit das Depotfett des Organismus mobilisiert zu werden und zur Leber zu wandern. Die Ketonkörperbildung bei Zufuhr von Hypophysenvorderlappenextrakt ist nach *Mirsky* (1936) an die Tätigkeit der Leber gebunden und unterbleibt nach Leberexstirpation.

Bei Behandlung mit Fettstoffwechselhormon konstatierten *Speitkamp* und *Anselmino* (1941) eine Steigerung des Sauerstoffverbrauchs, aber ein Sinken des respiratorischen Quotienten.

*Anselmino* und *Hoffmann* (1936) haben bei Versuchen an Hunden gefunden, dass grosse Insulindosen die regulatorische Bildung des Fettstoffwechselhormons nach Fettbelastung verhindern. Kleinere Insulindosen übten dagegen keine hemmende Wirkung auf die Mobilisierung des ketogenen Hormons aus. Eine Insulinüberdosierung kann auf diese Weise eine Störung des Fettstoffwechsels auslösen.

Es ist unsicher, ob das fettstoffwechselregelnde Hormon direkt wirkt, oder ob die Wirkung desselben von den Nebennieren vermittelt wird. *Long* und *Lukens* (1935) beobachteten nämlich, dass die Ketonkörperausscheidung bei pankreasdiabetischen Tieren nach Nebennierenexstirpation ebenso wie nach Hypophysektomie verschwindet. Auch die leberfettsteigernde Wirkung des ketogenen Hypophysenhormons bleibt aus, wenn

die Nebennieren entfernt werden, und die Ketonurie hervorru-  
fende Wirkung desselben hört bei den nebennierenlosen Tie-  
ren auf.

Nach *Soskin* (1936) entfaltet das fettstoffwechselregelnde  
Hormon seine Wirkung beim Übergang des Fetts in Zucker.  
Hierfür soll da die Mitwirkung der Nebennierenrinde erforder-  
lich sein. Die Ketonkörperbildung hängt mit der Verwandlung  
des Fetts in Zucker zusammen.

Wahrscheinlich sind das fettstoffwechselregelnde und das  
corticotrope Hypophysenvorderlappenhormon verschiedene  
Substanzen, da sie im Hypophysenextrakt chemisch voneinan-  
der getrennt werden können (*Anselmino* und *Hoffmann, Collip*  
u. a.).

Es ist auch nicht sicher, ob das fettstoffwechselregelnde Hor-  
mon die glykostatische und glykotrope Wirkung verursacht,  
welche dem glykostatischen bzw. glykotropen Faktor im Hypo-  
physenvorderlappen zugeschrieben wird. Nach *Neufeld* und  
*Collip* (1938) ist es dieselbe wirksame Substanz, nach *Ansel-  
mino* und *Hoffmann* handelt es sich um zwei verschiedene  
Substanzen. Diese Frage ist noch nicht geklärt.

## Die diabetogene Wirkung der Hypophyse

Eine Zusammenfassung der hormonalen Wirkungen der Hy-  
pophyse ergibt, dass viele Möglichkeiten für das Zustandekom-  
men eines dauernden Diabetes bestehen:

1. Durch Ausfall der Wirkung des pankreatotropen Hormons  
kommt es zu einer Inselatrophie, welche zur Entstehung eines  
Insulinmangeldiabetes führt.

2. Eine Störung im kontrainsulären neurohormonalen Re-  
flexbogen bewirkt einen Regulationsdiabetes von anderem  
Typus.

3. Der glykotrope Faktor verursacht bei Hyperfunktion  
durch Blockierung des Leberglykogens einen Diabetes mit her-  
abgesetzter Kohlehydrattoleranz.

4. Bei Ausfall der corticotropen Hormonwirkung der Hypo-  
physe tritt eine Nebenniereninsuffizienz mit mangelnder Cor-

linbildung ein. Hierdurch kommt es wahrscheinlich zu Störungen in Phosphorylierungsprozessen des Organismus mit Beeinträchtigung der Resorption und Verwertung von Kohlehydraten, wobei Diabetesformen verschiedener Art entstehen, abhängig davon, welche Phosphorylierungsprozesse gestört sind (vgl. IV. Abschnitt und S. 263).

5. Eine Überproduktion des Fettstoffwechselhormons führt zur starken Ketonkörperbildung und zu einem Diabetes.

6. Die übermässige Erzeugung des kohlehydratstoffwechselregelnden Hormons bewirkt einen gesteigerten Glykogenzerfall und dadurch einen Diabetes.

Das thymotrope Hormon, welches nach *Bomskow* mit dem Kohlehydratstoffwechselhormon identisch ist, verursacht einen schweren Diabetes, der gelegentlich des Wachstums auftritt. Die Altersfrequenzkurve der schweren Diabetesfälle vom O-Typus weist auch ein starkes Maximum während der Wachstumsjahre auf (vgl. S. 346).

Hieraus geht hervor, dass es unnötig ist, das Vorkommen eines bestimmten diabetogenen Faktors in der Hypophyse anzunehmen; ein Diabetes kann vielmehr durch die Wirkung oder das Zusammenwirken mehrerer bereits bekannter Hypophysenhormone zustande kommen. Die Klärung von Einzelheiten bezüglich der Genese der verschiedenen diabetischen Zustände gehört zu den Aufgaben der weiteren Forschung.

*Johns, O'Mulvenny, Potts und Laughton* (1927) beobachteten bei Versuchen an Hunden die Entwicklung eines Diabetes nach Injektion eines Extrakts aus Hypophysenvorderlappen von Ochsen. Schon 24 Stunden nach der ersten Injektion trat eine Blutzuckersteigerung mit Polyurie und Zuckerausscheidung ein, welche 14 Tage anhielt. Dann verschwand dieser Diabetes, und die Tiere waren in der folgenden Zeit gegen den angewandten Hypophysenextrakt resistent.

Ein Dauerdiabetes nach Behandlung mit Hypophysenvorderlappenextrakt wurde, ebenfalls bei Versuchen an Hunden, zuerst von *Young* (1928) festgestellt (s. Abb. 1 S. 12).

*Young* verwendete grössere Hypophysenmengen, 350 g frischen Hypophysenvorderlappens von Ochsen entsprechend, zur Bereitung des Extrakts. Dieser wurde intraperitoneal in

steigenden Dosen im Laufe von 25 Tagen mit 3tägiger Pause zwischen zwei Injektionen eingespritzt. Dabei entsteht zunächst ein transitorischer Diabetes, welcher nach 7—10 Tagen verschwindet, dann aber bei weiterer energischer Behandlung wiederkommt und nach 11—25 Tagen dauernd wird.

Nach Young geht die temporäre Diabetesform an einem bestimmten Punkt in die permanente über. Ist dies eingetreten, so verschwindet der Diabetes nicht wieder. Das Auftreten der permanenten Diabetesform geht mit einer Gewichtsabnahme einher, welche die Zunahme ablöst, die im Stadium der temporären Diabetesform auftritt. Während dieses Stadiums ist die Ketonkörperausscheidung gering. Wenn die permanente Diabetesform beginnt, setzt nach Young plötzlich eine starke Ketonurie ein, welche so hochgradig werden kann, dass sich in kurzer Zeit ein diabetisches Koma entwickelt.

Ein Dauerdiabetes weist in der Regel keine Tendenz zum Zurückgehen auf, sondern nimmt eher mit der Zeit zu, auch wenn er mit Insulin behandelt wird.

Ein hypophysärer, von diabetogenen Hormonen des Hypophysenvorderlappens hervorgerufener Diabetes entwickelt sich nach Young bei Versuchen an Hunden in verschiedenen Stadien:

1. *Latenzperiode*, 1—2 Tage, in welcher keine Diabetes-symptome zu beobachten sind, in der aber eine gewisse Unempfindlichkeit gegen Insulin besteht;

2. *temporäres Diabetesstadium*, in den nächsten 6—9 Tagen. Relative Insulinunempfindlichkeit. Leber glykogenreich;

3. *Refraktärperiode*. Blutzuckergehalt normal, nach wie vor Unempfindlichkeit gegen Insulin und weiterbestehender hoher Leberglykogengehalt.

Durch starke Steigerung der Hypophysenextraktmenge kann die Refraktärperiode abgebrochen werden, wobei sich das

4. *permanente Diabetesstadium* entwickelt. Dieses hält auch an, nachdem die Behandlung abgebrochen worden ist. Da verschwindet die Unempfindlichkeit gegen Insulin.

Nach Young unterscheidet sich ein durch Injektion von Vorderlappenextrakt hervorgerufener *Hypophysenvorderlappendiabetes* in mehreren Beziehungen von einem durch Pan-

kreasexstirpation verursachten *Pankreasdiabetes*. Beim hypophysären Diabetes ist oft die 2- bis 3fache Insulinmenge erforderlich, um die Glykosurie zu beherrschen. Dies kann jedoch wechseln. Beim Pankreasdiabetes ist die Insulinbehandlung absolut indiziert, ein Hypophysärdiabetiker kann dagegen auch bei reichlicher Ketonkörperausscheidung ohne Insulin lange Zeit leben. An insulinfreien Tagen nimmt ein Pankreasdiabetiker viel mehr ab als ein Hypophysärdiabetiker. Der Quotient Zucker-: Stickstoffausscheidung sowie die Ketonkörperausscheidung ist nach *Young* beim Hypophysenvorderlappendiabetes grösser als beim Pankreasdiabetes.

Beim Hypophysendiabetes ist die Zuckertoleranz eine schlechte, und über 90 % der zugeführten Kohlehydrate können im Urin ausgeschieden werden. Der respiratorische Quotient wird von Glykosezufuhr nicht gesteigert, und bei reiner Fettkost nehmen sowohl Glykosurie wie Ketonurie ab.

Beim experimentellen Diabetes sind indessen die Schwankungen zwischen verschiedenen Tieren so gross, dass es nicht möglich ist, irgendwelche bestimmte Kennzeichen oder Grenzen zwischen verschiedenen Diabetesformen als Hilfsmittel bei einer verfeinerten Diagnostik zu konstruieren.

## Das duodeno-pankreatische Regulationssystem

Im Zusammenhang mit der Kohlehydratresorption aus dem Darmkanal sorgt die Blutzuckerregulation auch für das Blutzuckergleichgewicht bei Nahrungszufuhr. Es muss daher ein mit dem zerebral-hypophysären Regulationssystem koordiniertes weiteres Regulationssystem geben, welches enger an die eigentliche Verdauungstätigkeit geknüpft ist. Diese Funktion ist die Aufgabe des *duodeno-pankreatischen Systems* (vgl. S. 10).

*Bayliss* und *Starling* wiesen 1902 die Bedeutung der Duodenalschleimhaut für die Sekretion des Pankreassafts nach. Sie nahmen das Vorkommen eines hormonal wirkenden »Sekretins« an, welches die Bildung von Pankreassaft auslöst. Durch Arbeiten von *Hammarsten*, *Jorpes* und *Ågren* ist das Sekretin heute in reiner Form dargestellt.

Die hormonale Wirkung der Duodenalschleimhaut auf das Pankreas lenkte die Aufmerksamkeit der Forscher auch auf das Verhalten der Duodenalschleimhaut beim Diabetes und auf den Einfluss derselben auf die Insulinproduktion.

*Munroe* (1906) beobachtete als erster beim Diabetes Veränderungen der Duodenalschleimhaut. *Workman* fand bei Obduktion von 21 Diabetikern 10 Fälle mit hypertrophischer Schleimhaut und vergrößerten Valvulae Kerkringi. *Moore* (1906) stellte aus Duodenalschleimhaut vom Schwein einen Extrakt dar, welcher die Zuckerausscheidung beim Diabetes günstig beeinflusste. *Brainbridge* konnte diese Wirkung nicht nachweisen.

*Ivy* und *Fischer* (1924) haben ebenfalls aus Magen- und Duodenalschleimhaut einen Extrakt gewonnen, der in gewissen Fällen eine insulinähnliche Wirkung hatte.

*Takacs* isolierte 1927 aus der Duodenalschleimhaut eine Substanz, welche gleichzeitig Blutzuckersenkung und gesteigerte Gallensekretion hervorrief. Ein hinreichend gereinigtes Sekretin fand dagegen *Still* und *Shipner* (1929) ohne Einfluss auf das glykoregulatorische System. Ebensowenig konnten *La Barre* und *Ägren* irgendeine hypoglykämisierende Wirkung eines gereinigten, aus Duodenalschleimhaut dargestellten Sekretins beobachten.

Dagegen erhielt *Heller* 1929 durch Digestion eines aus Duodenalmucosa gewonnen Rohsekretins mit Trypsin oder Pepsin eine von ihm als *Duodenin* bezeichnete Substanz, welche bei peroraler Verabreichung die alimentäre Hyperglykämie bei gleichzeitiger Kohlehydratkost herabsetzte. *Tangle* und *Than* fanden hierbei ein gleichzeitiges Steigen des respiratorischen Quotienten. Sie brachten daher die Wirkung des Duodenins in Beziehung zu einer gesteigerten Kohlehydratverbrennung.

*La Barre* und *Ledrut* (1934) konstatierten eine starke Blutzuckersenkung, wenn verdünnte HCl bei pankreasdiabetischen Hunden intraduodenal zugeführt wurde. Durch Fällung mit Trichloressigsäure gelang es *La Barre*, das aus der Duodenalschleimhaut gewonnene Sekretin in zwei Fraktionen zu zerlegen, die eine, das *Exkretin*, mit erhaltener sekretionsfördernder Wirkung, die andere, das *Inkretin*, mit hypoglykämisie-



rendem Effekt. *La Barre* fand auch wie *Heller*, dass man aus dem Sekretin durch Pepsindigestion eine Substanz mit Hypoglykämie hervorrufender Wirkung erhalten konnte. Es ist nicht sicher, ob das *Hellersche* Duodenin mit dem *La Barreschen* Inkretin identisch ist.

*La Barre* behandelte 10 Fälle von Diabetes mit Inkretin und stellte eine blutzuckersenkende Wirkung fest. Leider ist bei diesen Versuchen die Möglichkeit spontaner, mit der periodischen Lebertätigkeit zusammenhängender Blutzuckervariationen unberücksichtigt geblieben. Der hypoglykämisierende Effekt des Inkretins zeigte sich auch bei pankreasdiabetischen Hunden nach vollständiger Pankreasexstirpation, weshalb das Inkretin unabhängig vom Pankreas wirken können muss.

*De Barbieri* (1937) hat die Untersuchungen von *La Barre* fortgesetzt. Er konnte die hypoglykämisierende Inkretinsubstanz auf drei verschiedenen Wegen aus dem Sekretin gewinnen:

1. Fällung des Sekretins mit Trichloressigsäure nach *La Barre*;
2. Extraktion des Neutralsalzniederschlags des Sekretins mit saurem Alkohol;
3. Extraktion des Sekretins mit saurem Alkohol-Äther.

Bei der Pepsindigestion der wie oben dargestellten Fraktionen waren 1 und 2 gegen die Digestion resistent und behielten ihre hypoglykämisierende Wirkung über 24 Stunden. Die 3. Fraktion büßte dagegen ihre Wirkung schon nach 30 Minuten ein. *De Barbieri* unterschied daher zwei hypoglykämisierend wirksame, mit dem Sekretin aus der Duodenalschleimhaut gewonnene Substanzen, die R-Substanz und die L-Substanz, welche sich durch verschiedene Löslichkeit in Äther und verschiedene Widerstandsfähigkeit gegen proteolytische Digestion auszeichnen.

Die R-Substanz, welche binnen 30 Minuten von Pepsindigestion zerstört wird, nannte *de Barbieri* *Duocrin*.

Die L-Substanz widersteht dagegen der Digestion länger als 24 Stunden und ist wahrscheinlich mit dem *La Barreschen* Inkretin, womöglich mit dem *Hellerschen* Duodenin identisch.

*De Barbieri* untersuchte die Wirkung des Duocrins an 11 Fällen von Diabetes. Er fand bei 8 Fällen einen blutzuckersenkenden Effekt. Nach einer Latenzzeit von einigen Tagen begann der Blutzuckerspiegel zu sinken, und der maximale Effekt stellte sich nach 8—50 Tagen ein. Drei Fälle waren sowohl gegen Duocrin wie gegen die Insulinwirkung resistent. Bei den Fällen, wo das Duocrin wirkte, war die Herabsetzung der Glykosurie und Ketonkörperausscheidung markanter als die Beeinflussung der Hyperglykämie. *De Barbieri* meint, die Wirkung des Duocrins sei anhaltender als die des Insulins, und es handle sich möglicherweise um eine insulotrope Wirkung. Der Inkretineffekt kann dagegen nicht insulotrop sein, da *La Barre* eine blutzuckersenkende Inkretinwirkung auch bei pankreaslosen Hunden beobachtet hatte.

Eine vom Sekretin verschiedene insulotrope Substanz wurde 1932 von *Macallum* und *Laughton* durch eine spezielle Präparationsmethode aus Duodenalschleimhaut dargestellt. Die Forscher fanden, dass dieser Extrakt den normalen Nüchternblutzuckergehalt nicht beeinflusste, wohl aber bei alimentärer Hyperglykämie eine ausgeprägt blutzuckersenkende Wirkung hatte. Nach 7- bis 10tägiger peroraler Verabreichung blieb der Effekt mehrere Wochen bestehen, im Gegensatz zu dem des Duocrins, welcher bald vorüberging. Der *Macallum-Laughtonsche* Extrakt hatte keine Wirkung auf den Blutdruck oder auf die Sekretion von Pankreassaft. Beim *Sandmeyerschen* Diabetes nach partieller Pankreasexstirpation bei Hunden beeinflusste dieser Extrakt nur die alimentäre Hyperglykämie. *Duncan* (1932), *Shumway*, *Williams* und *Fetter* (1935) haben den *Macallum-Laughtonschen* Extrakt an 30 Fällen von Diabetes klinisch geprüft:

Von 12 schweren Fällen reagierten 8 günstig auf den Extrakt, 4 waren resistent, von 18 leichten Fällen wurden 11 günstig beeinflusst, 7 waren resistent. Die vier schweren Diabetesfälle, welche gegen den *Macallum-Laughtonschen* Extrakt resistent waren, waren deutlich insulinüberempfindliche Fälle. *Flint* und *Michaud* haben die Wirkung des *Macallum-Laughtonschen* Extrakts eingehend analysiert und festgestellt, dass es sich um einen stabilisierenden Faktor handelt, der

synergistisch mit Insulin, aber antagonistisch gegen den Einfluss der Hypophyse wirkt.

Bei der Darstellung des *Macallum-Laughtonschen* Extrakts erhielt *Macallum* bisweilen ein Präparat, dem die blutzuckersenkende Wirkung abging oder das sogar anhaltende Blutzuckersteigerung bewirkte, welche sich bei gleichzeitiger Verschlimmerung der Kohlehydrattoleranz über 1—2 Monate erstrecken konnte. Wenn Insulindosen, welche in gewöhnlichen Fällen wirksam waren, gleichzeitig mit diesem paradox wirkenden *Macallum-Laughtonschen* Extrakt gegeben wurden, war die Wirkung des Insulins merklich abgeschwächt oder blieb ganz aus. Der *Macallum-Laughtonsche* Extrakt konnte daher eine Fraktion mit *insulinantagonistischer* Wirkung enthalten. *Macallum* fand 1938, dass diese insulinantagonistisch wirkende Substanz nicht nur in der Duodenalschleimhaut vorkommt, sondern auch, und zwar regelmässig und reichlicher, im Pankreasgewebe, aus dem sie leicht zu gewinnen ist. Diese aus Pankreas dargestellte Substanz ist mit der insulinantagonistisch wirkenden Substanz aus dem Duodenum identisch.

Den normal wirksamen *Macallum-Laughtonschen* Extrakt erhält man am besten bei Tieren, welche eben gefressen haben. Lässt man die Tiere vor der Präparierung der Duodenalschleimhaut 48 Stunden hungern, so erhält man einen sehr schwach wirkenden Extrakt. Die wechselnde Menge der wirksamen Substanz im tätigen oder ruhenden Duodenum beruht nach *Macallum* darauf, dass die Substanz nicht im Zwölffingerdarm sondern in der Bauchspeicheldrüse gebildet und der Duodenalschleimhaut mit dem Pankreassaft zugeleitet wird. *La Barre* beobachtete auch, dass intraduodenal zugeführte schwache Säure nur dann eine Blutzuckersenkung veranlasste, wenn gleichzeitig Pankreassaft sich im Duodenum befand.

Nach anhaltendem Hungern wird der Organismus gegen Kohlehydrate empfindlicher, und die postalimentäre Hyperglykämie wird dabei stärker als normal. Ein wahrscheinlich hiermit zusammenhängendes Phänomen ist die von *Åkerrén* nachgewiesene Erscheinung, dass die Nüchternblutzuckerwerte sinken, wenn nicht an Diabetes leidende Personen einige Tage

lang grosse Kohlehydratmengen zu sich nehmen. Beim Diabetes bleibt dieser Äkerrén-Effekt aus. Bei Glykosebelastung nach vorausgehender kohlehydratarmer Kost wird auch die Hyperglykämie stärker als nach kohlehydratreicherer Ernährung.

Nach *Flint* (1938) ist die gesteigerte Kohlehydratempfindlichkeit nach vorangehender Kohlehydratkarenz auf einen Ausfall der blutzuckersenkenden Faktoren des Pankreassafts zurückzuführen.

Die aus der Bauchspeicheldrüse nach *Macallum-Laughton* gewonnene insulinantagonistische Substanz erhält nach saurer Hydrolyse eine entgegengesetzte Wirkung und wird synergistisch mit Insulin. Ob diese Substanz mit der insulotropen Substanz des aus Duodenalschleimhaut dargestellten *Macallum-Laughtonschen* Extrakts identisch ist, ist noch nicht bekannt.

*Rathery, Bierry* und *Traverse* haben 1936 auch aus der Jejunalschleimhaut eine hypoglykämisierend wirkende Substanz dargestellt.

Aus Pankreas und Duodenum sind also bisher folgende hormonalen Substanzen gewonnen worden, welche in die Blutzuckerregulation und den Kohlehydratstoffwechsel eingreifen:

1. *Insulin*. Blutzuckersenkend.
2. *Macallum-Laughtonsche Substanz*, wahrscheinlich insulotrop, die Inseln zu gesteigerter Insulinbildung stimulierend.
3. *Insulinantagonistische Substanz*. Blutzuckersteigernd.
4. *Inkretin* = L-Substanz. Blutzuckersenkend.
5. *Duocrin* = R-Substanz. Möglicherweise insulotrop.

Es ist nicht sicher, ob Inkretin und Duocrin aus dem Sekretin stammen, oder ob es Substanzen sind, welche dasselbe begleiten. Jedenfalls scheint das reine Sekretin keine blutzuckersenkende Wirkung zu besitzen.

*Das duodeno-pankreatische Hormonsystem wird von der Verdauungstätigkeit und der mit dieser verbundenen Reizung des Zwölffingerdarms in Gang gebracht. Bei der normalen Blutzuckerregulation muss dasselbe mit den übrigen glykoregulatorischen Systemen koordiniert sein.*

## VII. ABSCHNITT

Die physikalisch-chemische Regulation  
beim Diabetes.

Die Vorgänge beim Stoffwechsel lassen sich grundsätzlich in zwei Hauptgruppen einteilen: 1. *substanzverbrauchende, Energie frei machende Prozesse* (Energienstoffwechsel), 2. *regulatorische Prozesse* von nicht energetischer Art (Regulationsstoffwechsel).

Der *Energienstoffwechsel* stellt die Energie zur Verfügung, welche für den Fortgang der Lebensprozesse und für die Aufbauarbeit im Organismus gebraucht wird. Diese Energiequelle ist die *Conditio sine qua non* für die Lebensfunktionen der Körperzellen, für das Wachstum derselben und für die normale Produktion der Körpersubstanz.

Der *Regulationsstoffwechsel* dagegen hat für die Aufrechterhaltung des physikalisch-chemischen Gleichgewichtszustandes in den Körpersäften und für die Bildung der Ausscheidungs- und Abfallprodukte zu sorgen, welche für das ungestörte Fortbestehen dieses Gleichgewichts notwendig sind.

Sollen die Lebensprozesse reibungslos, normal verlaufen, so muss eine bestimmte Relation im Substanztausch zwischen Körpersäften und zelligen Elementen bestehen. Diese wechselt je nach den verschiedenen Funktionen der Zellen und nach dem funktionellen Zustand derselben.

Die Aminosäuren, die Bausteine der Eiweisskörper, sind amphotere Elektrolyte. Ihre Ladung hängt von der Ionenkonzentration des umgebenden Mediums ab. Die kolloide Ladung der Eiweissmoleküle ist daher gegen Veränderungen dieser Konzentration der Gewebssäfte hochgradig empfindlich. Eine grössere Veränderung der Ladungsverhältnisse kann zu einer Ausflockung der kolloiden Substanz führen. Diese findet z. B.

bei einer Wasserstoffionenkonzentration statt, welche dem isoelektrischen Punkt der Eiweisskörper entspricht.

Kommt es in einer lebenden Zelle zu einer solchen Ausflockung, so tritt ein katastrophaler Zustand ein: der Lebensprozess in der Zelle kommt zum Stehen, und bei grösserem Umfang dieses Vorgangs droht dem ganzen Organismus ein plötzlicher Tod. Eine Änderung der elektrostatischen Ladung der Zellkolloide kann zu anaphylaktischen Zuständen führen.

Der Substanz Austausch zwischen Zellen und umgebenden Gewebssäften ist an die *Permeabilität der zellbegrenzenden Membran, den Bau und die Funktion derselben* gebunden. Dadurch erhält der Zustand der Zellmembran eine entscheidende Bedeutung für das normale Leben der Zelle.

Die Struktur der Zellmembran ist wahrscheinlich die eines Mosaiks, mit orientierten Lipoid- und Eiweissmolekülen, die sich durch Wirkung verschiedener Ionen in der Umgebung und der Zellstruktur bei normalen Verhältnissen im elektrostatischen Gleichgewicht befinden und dadurch der lebenden Zelle eine bestimmte Ladung verleihen. Durch die Lebensfunktionen der Zelle und durch den Stoffwechsel steht die Struktur der Zellmembran in einem ständigen Wechsel, wodurch der Substanz Austausch stattfindet. Je intensiver der Stoffwechsel der Zelle ist, desto ausgiebiger ist der Substanz Austausch. Die Harnbildung im Zusammenhang mit dem starken Sauerstoffverbrauch der Nieren und dem oxydativen Stoffwechsel derselben ist ein Beispiel hierfür.

Ein für die physikalisch-chemische Regulation und die Aufrechterhaltung des Ionengleichgewichts im Organismus bedeutungsvoller mitwirkender Faktor ist der Mineral- und Wasserumsatz. Dieser steht wie der Kohlehydratstoffwechsel unter neurohormonalem Einfluss.

Abgesehen von der chemischen Zusammensetzung muss in den Körperflüssigkeiten noch ein Gleichgewicht in bezug auf u. a. Wasserstoffionenkonzentration, osmotischen und kolloidosmotischen Druck sowie physiologische Ionenrelation vorliegen.

Das Säuren-Basengleichgewicht im Organismus ist von allergrösster Bedeutung für den normalen Ablauf der Zellfunktion.

nen. Der Frage der physikalisch-chemischen Regulation der Wasserstoffionenkonzentration muss beim ketosäurebildenden Diabetes ganz besondere Aufmerksamkeit gewidmet werden.

## Die Wasserstoffionenregulation

Elektrolyte sind in Lösung teilweise oder gänzlich in freie, elektrisch geladene Ionen dissoziiert: positive *Kationen* und negative *Anionen*. Nach dem Gesetz der Massenwirkung befindet sich das Produkt dieser Ionen in einem Gleichgewichtszustand mit dem nicht dissoziierten Elektrolytrest. Die Dissoziationskonstante ändert sich mit der Temperatur.

Charakteristisch für Säuren ist die Abspaltung freier positiv geladener *Wasserstoffionen*, mit  $[H^+]$  bezeichnet. Basen dagegen spalten negativ geladene *Hydroxylionen*,  $[OH^-]$ , ab. All das beruht auf einer Elektronenverschiebung im Molekül.

Das reine Wasser ist ein amphoterer Elektrolyt mit sehr geringem Dissoziationsgrad. Hier ist die Anzahl der Wasserstoff- und Hydroxylionen dieselbe, weshalb das Wasser neutral reagiert. Auf Grund der schwachen Dissoziation kann  $H_2O$  als im Verhältnis zu  $[H^+]$  und  $[OH^-]$  konstant erachtet werden, und infolgedessen nimmt die Gleichgewichtsgleichung, die Dissoziationskonstante, des Wassers die Form

$$K_{H_2O} = [H^+] \cdot [OH^-]$$

an.

Nach *Sørensen* ist die Dissoziationskonstante des Wassers  $K_{H_2O}$ , bei:

$$18^\circ C: 0,74 \cdot 10^{-14} = 10^{-14,13}$$

$$25^\circ \text{ »: } 1,27 \cdot 10^{-14} = 10^{-13,90}$$

$$37^\circ \text{ »: } 3,13 \cdot 10^{-14} = 10^{-13,51}$$

Da die Anzahl der Wasserstoff- und Hydroxylionen hier die gleiche ist, wird

$$K_{H_2O} = [H^+]^2$$

woraus folgt, dass

$$[H^+] = \sqrt{K_{H_2O}}$$

ist.

Die Wasserstoffzahl für reines Wasser wird daher nach Sørensen:

$0,86 \cdot 10^{-7}$	bei $18^\circ$ ,	pH 7,07	entsprechend,
$1,13 \cdot 10^{-7}$	» $25^\circ$ ,	pH 6,95	»
$1,77 \cdot 10^{-7}$	» $37^\circ$ ,	pH 6,75	»

Dies entspricht dem Neutralpunkt. Eine Lösung wird sauer, wenn die Anzahl der Wasserstoffionen pro Liter grösser ist als im reinen Wasser, und mit steigender Wasserstoffzahl sinkt der pH-Wert. Ist die Anzahl der Wasserstoffionen kleiner oder die der Hydroxylionen grösser als im reinen Wasser, so wird die Lösung alkalisch. Der pH-Wert steigt dabei mit sinkender Wasserstoffzahl oder wachsender Hydroxylzahl.

Die Stärke einer Säure hängt von der Dissoziationskonstanten derselben ab. Je höher diese ist, d. h. je vollständiger die Dissoziation ist, desto grösser ist die Anzahl der pro Volumeneinheit vorhandenen Wasserstoffionen, und desto stärker ist die Säure.



(ii)

Wird einer schwächeren Säure eine stärkere zugesetzt, so steigt die Konzentration ( $\text{H}^+$ ), wobei das Gleichgewicht verschoben und die schwächere Säure noch schwächer dissoziiert wird. Eine schwache Säure wirkt dadurch als eine *Puffer-substanz* regulierend auf die Wasserstoffionenkonzentration ein. Die Wirkung wird noch verstärkt, wenn die schwache Säure ganz oder zum Teil durch eines ihrer Alkalisalze ersetzt wird. Durch Wahl geeigneter Puffersubstanzen in passender Menge lässt sich eine so starke *Pufferwirkung* erzielen, dass eine Lösung bei konstantem pH gehalten werden kann, und zwar auch bei Zusatz von sauren oder basischen Substanzen.

Bei der biologischen Wasserstoffionenregulation spielt die Pufferwirkung eine sehr wichtige Rolle. Bedeutungsvolle Puffersubstanzen im Organismus sind u. a. primäre und sekundäre Phosphate, Bikarbonat und Karbonat.

Die Dissoziationskonstanten sind für:



Primäres (Mono-) Phosphat, $\text{BH}_2\text{PO}_4$ , bei 25°:	$1,1 \cdot 10^{-2}$ ; $\text{pK}_1$ 1,95
Sekundäres (Bi-) Phosphat, $\text{B}_2\text{HPO}_4$ , " "	$1,95 \cdot 10^{-7}$ ; $\text{pK}_2$ 6,7
Bikarbonat, $\text{BHCO}_3$ , " "	$3,04 \cdot 10^{-7}$ ; $\text{pK}_1$ 6,52
Karbonat, $\text{B}_2\text{CO}_3$ , " "	$6,0 \cdot 10^{-11}$ ; $\text{pK}_2$ 10,22

Das Mass für die Menge freier Säure ist die *Titrations- oder Gesamtazidität*. Diese wird durch Zusatz einer äquivalenten Alkalimenge bis derjenige pH-Wert erreicht ist, welcher dem gebildeten Neutralsalz und dem Farbumschlag des mit Rücksicht hierauf gewählten Indikators entspricht.

Die *aktuelle Reaktion oder Ionenazidität* hat dagegen nicht mit der Gesamtsäuremenge zu schaffen, sondern wird von dem Verhältnis zwischen freier Säure und Salz, also von dem Dissoziationsgrad, bestimmt.

$$[\text{H}^+] = K_1 \frac{[\text{BH}_2\text{PO}_4]}{[\text{B}_2\text{HPO}_4]} = K_2 \frac{[\text{H}_2\text{CO}_3]}{[\text{BH} \cdot \text{CO}_3]} = K_3 \frac{[\text{BH} \cdot \text{CO}_3]}{[\text{B}_2 \cdot \text{CO}_3]}$$

Einen Massstab für die Pufferungsfähigkeit eines Systems bildet die Änderung des pH-Werts beim Zusatz einer bestimmten Menge Säure oder Base. Bei Zusatz von Säure bzw. Alkali zu einer Lösung mit Pufferwirkung werden die Elektrolytenkomponenten des Puffersystems mehr oder weniger stark dissoziiert, so dass der pH-Wert der Lösung konstant wird. Je besser die Pufferwirkung ist, desto mehr Säure oder Alkali kann zugesetzt werden, ohne dass sich die Wasserstoffionenkonzentration ändert. Die *Pufferkapazität oder Moderationsbreite* eines gepufferten Systems entspricht dem Säuren- bzw. Basenäquivalent, welches zugesetzt werden muss, damit die Pufferung gleich Null werde. Die Pufferkapazität ändert sich mit der Konzentration der Puffersubstanzen.

Bei steigender Phosphat- oder Bikarbonatmenge nimmt die Moderationsbreite des gepufferten Systems und damit die Fähigkeit, saure Produkte zu neutralisieren, zu.

Die *Reaktionsregelung des Organismus* ist einerseits an die eigene Pufferung des Blutes und der Gewebe gebunden und hängt andererseits mit der Wegschaffung störender Substanzen und der Entfernung derselben aus dem Blute zusammen. Es ist die Belastung des Regulationssystems als Ganzes, welche

für den Zustand des Organismus und die säuren-basenregulierende Fähigkeit desselben den Ausschlag gibt. Die Wasserstoffionenkonzentration des Blutes kann auch bei Störungen des Säuren-Basengleichgewichts innerhalb normaler Grenzen liegen, solange der Regulationsmechanismus nicht überbelastet ist. Die Störung ist da kompensiert.

Der pH-Wert des venösen Blutes beträgt nach *Michaelis* normalerweise etwa 7,59 bei 18°, was 7,38 bei 38° entspricht. Die Differenz zwischen arteriellem und venösem Blut beläuft sich auf höchstens 0,14.

Bei einem Diabetes mit Bildung organischer Säuren ändert sich sogar in einem praekomatösen Stadium der pH-Wert des Blutes nur wenig. Erst während der Endstadien des Komas kann der pH-Wert auf bis gegen 6,9 absinken.

Die Hauptfaktoren bei der Säuren-Basenregulation des Organismus sind das Ausscheidungsvermögen der Nieren, die Pufferung des Blutes und der respiratorische Gaswechsel.

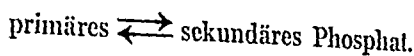
## Nierentätigkeit und Reaktionsregelung

Die Ausscheidung saurer und basischer Substanzen gibt den Nieren die Möglichkeit, in die Regulation der Reaktion des Blutes kräftig einzugreifen. Durch Ausscheidung des Überschusses im Urin kann der Gehalt des Blutes an diesbezüglichen Stoffen verhältnismässig konstant gehalten werden. Wie vollständig die Nieren überflüssige Substanzen zu eliminieren vermögen zeigt Abb. 75 (S. 307), in der ein Beispiel von Brenztraubensäureausscheidung im Urin bei praktisch konstantem Brenztraubensäuregehalt des Blutes gegeben wird.

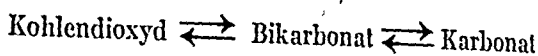
Durch die wechselnde Ausscheidung saurer Substanzen wird der Säuregrad des Urins beträchtlichen Variationen unterworfen. Der pH-Wert desselben wechselt daher normalerweise zwischen 5,0 und 7,2, abhängig von der Nahrung und anderen Faktoren. *Die normale Variationsbreite der Harnazidität ist die Voraussetzung für ein konstantes Säuren-Basengleichgewicht im Blute.* Hierbei ist eine ungestörte Nierenfunktion von allergrösster Bedeutung.

## Der Urin als Pufferlösung

Da der Urin Phosphate und Alkalisalze schwach dissoziierter Säuren enthält, besitzt derselbe eine gewisse Pufferwirkung, welche die Ionenkonzentration ausgeschiedener saurer Produkte modifiziert. In saurem Urin ist das vorherrschende Puffersystem:

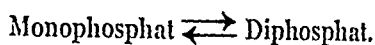


Ist der Harn alkalisch, so wird daneben das System:



zu einem wichtigen Puffer.

Den Variationen der Ionenazidität im sauren Urin entspricht die Verschiebung:



In stark saurem Harn sind bis 90 % des Gesamtphosphors in Form primären Phosphats vorhanden. Die Ausscheidung starker Säuren erfolgt stets zusammen mit einer äquivalenten Menge Alkali, welches hauptsächlich als Monophosphat gebunden ist. Da die Phosphorsäure im Blut zu etwa 85 % als Dinatriumphosphat gebunden ist, die sauren Valenzen aber als Monophosphat ausgeschieden werden, sparen die Nieren die Hälfte des Alkaligehalts des Blutes für den Organismus.

Je mehr saure Substanzen ausgeschieden werden müssen, desto grösser sind die hierzu erforderlichen Phosphatmengen, und um so mehr wird der pH-Wert des Urins nach der sauren Seite verschoben. Mit steigenden Phosphatmengen nimmt auch die Pufferkapazität des Harns und damit die Fähigkeit desselben zu, die sauren Stoffwechselprodukte zu eliminieren, ohne dass sich der pH-Wert in höherem Grade zu ändern braucht. Tab. 10 macht die Pufferungsfähigkeit zweier verschiedener Urine, eines mit pH 5,2 und eines anderen mit pH 6,3, ersichtlich. Im ersteren Falle ist die Pufferungsfähigkeit des Urins ziemlich gross, im letzteren ganz gering. Bei einem hohen pH-Wert ist keine nennenswerte Pufferungsfähigkeit des Urins

erforderlich, da schon von vornherein ein Basenübergewicht im Harn vorliegt. Zusatz von Säure verändert da den pH-Wert des Urins sehr stark.

TAB. 10.

Pufferungsvermögen des Urins bei verschiedenem pH.

	Urin I pH 5,2				Urin II pH 6,3			
		pH		pH		pH		pH
100 ml Urin + 2 ml N/10	NaOH	5,32	HCl	5,02	NaOH	6,8	HCl	5,92
» » » + 4 » »	»	5,55	»	4,92	»	7,45	»	5,60
» » » + 6 » »	»	5,85	»	4,82	»	8,3	»	5,35
» » » + 8 » »	»	6,20	»	4,78	»	8,5	»	4,92
» » » + 10 » »	»	6,60	»	4,70	»	9,8	»	4,70
» » » + 12 » »	»	8,15	»	4,60	»	—	»	4,12
» » » + 14 » »	»	—	»	4,40	»	—	»	3,60
» » » + 16 » »	»	—	»	4,05	»	—	»	—

*Das Studium der im Urin ausgeschiedenen Produkte gewährt bei ungeschädigter Nierenfunktion die Möglichkeit, Störungen im Verlauf des Zwischenstoffwechsels zu verfolgen. Im Urin findet man zum grossen Teil die im Körper gebildeten, aber nicht weiterumgesetzten Intermediärprodukte. Ausscheidungsdiagramme bieten daher einen Weg zum direkten Studium der Stoffwechselstörung.*

### Ketonkörperausscheidung und hämorenaler Ketosäureindex

Bei Störungen des Fettsäureumsatzes ist das Studium der Ketonkörperausscheidung von speziellem Interesse. Dies gilt namentlich für die  $\beta$ -Oxybuttersäure, welche eine nichtflüchtige Substanz ist. Bei ungeschädigter Nierenfunktion findet man die  $\beta$ -Oxybuttersäure, welche im Organismus nicht weiterumgesetzt wird, zum grössten Teil im Harn wieder. Azeton und Diazetsäure sind dagegen flüchtigere Substanzen, welche teilweise in der Respirationsluft ausgeschieden werden.

Nach *Engfeldt* sind im Blut normalerweise bis 0,15 mg% Gesamtazeton (Azeton-Diazetsäure) und bis 1,5 mg%  $\beta$ -Oxybuttersäure vorhanden. Bei mässiger Azidose steigt der  $\beta$ -Oxybuttersäuregehalt des Blutes auf 20–70 mg% und kann in schweren Fällen mehrere hundert mg% erreichen. *Engfeldt* hat Werte von bis 243 mg% gefunden, *Elmer* und *Scheps* bis 316 mg% und *Fazeka* sogar bis 565 mg%.

Bei ungeschädigten Nieren soll nach *Abraham* und *Altmann*

$$\text{der hämorenale Ketonindex} = \frac{\text{Harnkonzentration}}{\text{Blutkonzentration}}$$

für das Gesamtazeton bei etwa 10–12 liegen, im Praekoma und Koma aber infolge mangelhafter Ausscheidung durch die geschädigten Nieren auf ungefähr 1–2 sinken. Für die  $\beta$ -Oxybuttersäure beträgt der entsprechende Indexwert nach *Engfeldt* bei ungeschädigten Nieren 6–7, er kann aber im Praekoma und Koma mit zunehmender Niereninsuffizienz auf Werte unter 1 fallen. Dabei kann die ausgeschiedene Ketonkörpermenge trotz hochgradiger Ketonämie verhältnismässig klein sein.

Zwischen Ketonämie und Ketonkörperausscheidung besteht jedoch keine feste Korrelation, und der hämorenale Ketonindex wechselt seinem numerischen Wert nach in sehr hohem Grade. *Snapper* hat einen Fall von Diabetes mit einer 90 mg% entsprechenden Ketonämie ohne nachweisbare Ketonkörperausscheidung im Urin beschrieben. Im Schrifttum kann man Fälle von diabetischem Koma mit negativer *Gerhardtscher* Reaktion im Harn finden (*Bock, Field* und *Adair, Mac Caskey* u. a.). *Elmer* und *Scheps* haben über einen Diabetesfall berichtet, bei welchem der Ketonindex trotz zunehmender Azidose von 1,6 auf 9,7 stieg. In diesem Falle sank zwar die Ketonämie von 236,7 mg% auf 28,2 mg%, aber die Alkalireserve blieb niedrig, und nach 12 Stunden kam ein Koma diabeticum zum Ausbruch.

Der hämorenale Ketonindex wechselt auch bei ein und demselben Kranken an verschiedenen Zeitpunkten und ist daher ebenfalls keine individuell konstante Grösse. Die hier einge-

fügte Tab. 11 macht die Schwankungen des hämorenalen Ketonindexes während des Tages bei drei verschiedenen Diabetespatienten im Hunger ersichtlich.

Tab. 11.  
Hämorenaler  $\beta$ -Oxybutter-säureindex bei 3 Fällen  
von Diabetes im Hunger.

Zeit	Fall I			Fall II			Fall III		
	$\beta$ -Oxybutter-säure mg %		hämorenaler Index	$\beta$ -Oxybutter-säure mg %		hämorenaler Index	$\beta$ -Oxybutter-säure mg %		hämorenaler Index
	Blut	Urin		Blut	Urin		Blut	Urin	
8--10		195			290			< 5	
9	18		10,9	2 <sup>1</sup>		101	285		
10--12	16	80	5,2	2	335	221	-	Sp.	< 0,3
12--14	30	260	8,8	2	255	170	289	Sp.	0,02
14--16	6	295	10,0	2	260	173	-	Sp.	
16--18	38	175	4,6	279	385	1,4	126	Sp.	
Ammoniak-ausscheidung, 8--18 Uhr	175 ml N/10			626 ml N/10			115 ml N/10		

<sup>1</sup> Weil der  $\beta$ -Oxybutter-säuregehalt nicht erhöht war, und die angewandte Methode für kleine Mengen  $\beta$ -Oxybutter-säure nicht sichere Werte liefert, wird ein höchster normaler Wert angegeben.

Erster Fall (I im Tab. 11) v. S. 285, Fall 12 Ehn K., Ausscheidungsdiagramm dort wiedergegeben. Die beiden übrigen Fälle, II (Gösta M.) und III (Axel T.), zeigen folgende klinischen Bilder:

Fall 2. Gösta M., geb. 1904.

Keine erbliche Veranlagung für Diabetes.

1916 Appendicitis mit Peritonitis, sonst stets gesund gewesen. Im Oktober 1928 plötzlich einsetzende Müdigkeit und Durstgefühl ohne bekannte Ursache. Wog 78 kg, magerte nicht ab. Der Diabetes wurde nach 11 Tagen konstatiert. Mässige Diät mit 100 g Brot ohne Insulin. Zeitweise zuckerfrei. Jetzt langsame Abmagerung. Im Februar 1940 ein Schwindelanfall, im Anschluss daran Ketonurie, weshalb eine Insulinbehandlung eingeleitet wurde. Später munter, aber ab und zu Schwindel, bis 15. II. 1942. Pat. stürzte da beim Skilaufen, schlug sich am Rücken und wurde im Laufe des Tages viermal bewusstlos. 20. II. 1942 Länge 172 cm, Gewicht 61,6 kg. Pulsfrequenz 69, Blutdruck im Liegen 110/70, im Stehen 70/2 (nicht messbar). Herz o. B., Reflexe und Augenhintergrund auf beiden Seiten normal. Innere Organe o. B.

Der Diabetestypus geht aus Abb. 12 hervor.

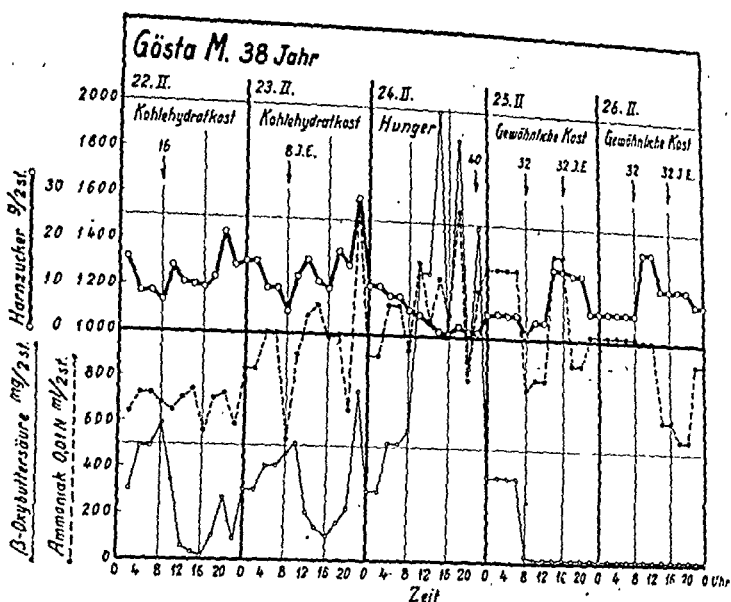


Abb. 42. Ausscheidungsdiagramm bei Diabetes mit Ketonurie. Starke Ammoniakbildung, welche auch mit steigender Ketonurie bei Verminderung des Insulins zunimmt. Während des Hungertages (24. II.) grosse  $\beta$ -Oxybutter-säureausscheidungswellen mit ungenügender Ammoniakbildung. Um etwa 20 Uhr Übelkeit und gesteigertes Durstgefühl, weshalb um 21.30 Uhr Insulin gegeben wurde. Vorher am Tage Allgemeinzustand o. B.

Fall. 3. Axel T., geb. 1917.

Kein Diabetes in der Familie.

1926 Pneumonie mit linksseitigem Empyem, sonst stets gesund gewesen. Im Dezember 1941 leichte Racheninfektion mit geringer Temperatursteigerung. Lag nicht zu Bett. Nach 14 Tagen zunehmender Durst

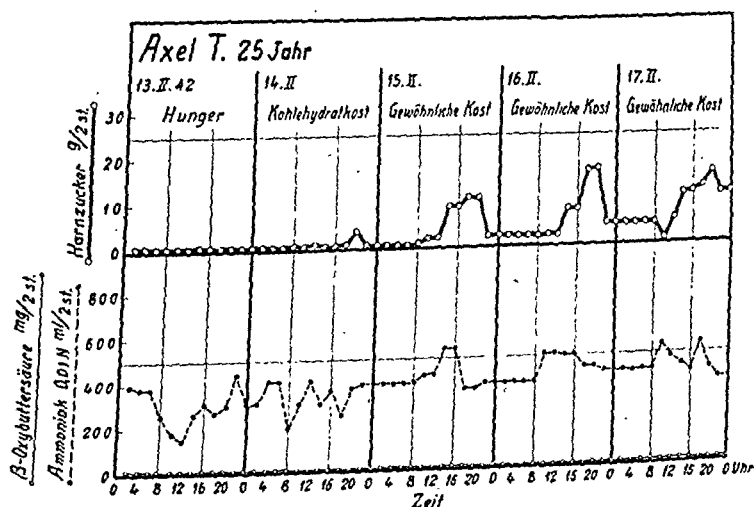


Abb. 43. Ausscheidungsdiagramm bei Diabetes mit Ketonämie, aber ohne Ketonurie.

und Müdigkeit. Unerhebliche Abmagerung. Der Diabetes wurde Mitte Januar 1912 festgestellt und die Insulinbehandlung unmittelbar eingeleitet. 10. II. 1912 Länge 176 cm, Gewicht 61,4 kg. Pulsfrequenz 56, Blutdruck 120/70. Herz: systolisches Geräusch, Grenzen normal. Innere Organe sonst o. B.

Der Diabetestypus ergibt sich aus Abb. 43.

Da die Ketonkörper in grossem Ausmass in den Nieren verbrannt werden, spiegelt sich in dem hämorenalen Ketonindex nicht nur die Ketonkörperausscheidungsfunktion der Nieren wider, sondern auch die Verbrennung der Ketonkörper in diesem Organ.

1. Ist die Ketonkörperbildung im Organismus eine starke, aber die Ketosäureverbrennung in den Nieren nicht gesteigert und die Ausscheidungsfunktion für Ketonkörper normal, so kommt bei ungeschädigten Nieren eine der Ketonämie entsprechende Ketonurie zustande (Fall 1 der vorstehenden Tab. 11).

2. Wenn die Ausscheidungsfunktion der Nieren sehr ausgiebig und die Ketonkörperverbrennung verstärkt ist; kann es zu einer so hochgradigen Ketonurie kommen, dass der Ketonkörpergehalt des Blutes normal wird, wobei der hämorenale Index sehr hoch wird (Fall 2). Beachtenswert ist die starke Ammoniakausscheidung bei diesem Falle.

Wenn in diesem Falle statt der  $\beta$ -Oxybuttersäure die Azeton-Diazetsäure bestimmt wird, erscheint ein anderer Index (Tab. 12).

TAB. 12.  
Hämorenaler Gesamtazetonindex.

Fall 2 Güsta M. 38 J.	Hunger 24. II.			
	Blut- zucker mg %	Gesamtazeton		hämo- renaler Index
		Blut mg %	Urin mg %	
8—10	378	28	81	2,9
10—12	268	21	68	3,2
12—14	259	13	56	4,3
14—16	259	17	87	5,1
16—18	233	19	97	5,1

Azeton und Diazetsäure sind also in diesem Falle im Blut in erhöhter Menge vorhanden, nicht aber  $\beta$ -Oxybuttersäure.



3. Ist dagegen die Ketonkörperausscheidungsfunktion ganz unzulänglich und die Ketonkörperverbrennung nicht verstärkt, so entwickelt sich eine Ketonämie hohen Grades, aber die Ketonkörperausscheidung wird sehr gering und der hämorenale Index dadurch niedrig (Fall 3). Hier ist auch die Ammoniakausscheidung gering.

Wenn im letzten Falle Nahrung zugeführt wird, bessert sich die Kohlenhydratverbrennung und die Ketonämie wird geringer. Gleichzeitig wird der Blutzuckergehalt höher und die Zuckerdiurese vermehrt, was aus Abb. 43 und der hier folgende Tab. 13 von demselben Patienten hervorgeht.

TAB. 13.

Hämorenaler  $\beta$ -Oxybuttersäureindex im Hunger und bei Nahrungszufuhr.

Fall 3 Axel T. 25 J.	Hunger 13.II.				Gewöhnl. Kost 17.II.			
	Blut- zucker mg %	$\beta$ -Oxybuttersäure mg %		hämo- renaler Index	Blut- zucker mg %	$\beta$ -Oxybuttersäure mg %		hämo- renaler Index
		Blut	Harn			Blut	Harn	
8—10	—	—	—	< 0,007	244	166	< 2	< 0,03
9	174	285	< 2		296	131	Sp.	
10—12	144	—	Sp.		358	36	Sp.	
12—14	143	289	Sp.		301	41	Sp.	
14—16	148	—	Sp.		341	47	Sp.	
16—18	132	426	Sp.					
Ammoniak- ausscheidung 8—18 Uhr	115 ml N/10				242 ml N/10			

Der hämorenale Index ist infolgedessen völlig von der Nierenfunktion abhängig und wechselt von Fall zu Fall innerhalb weiter Grenzen, und zwar auch bei ein und demselben Individuum an verschiedenen Zeiten. Man muss dabei prinzipiell zwischen der Ketonkörperverbrennungs- und Ketonkörperausscheidungsfunktion der Nieren unterscheiden, welche beide den hämorenalen Ketosäureindex in hohem Masse beeinflussen. Die Ketonkörperverbrennung scheint mit der Ammoniakausscheidung zusammenzuhängen.

## Die Ammoniakbildung

Im ruhenden Muskel hat *Meyerhof* eine Ammoniakbildung bei Abwesenheit von Sauerstoff gefunden. *Embden* und *Parnas* haben auch eine Ammoniakbildung während der anaeroben Phase der Muskelarbeit nachgewiesen. Dabei konnte *Parnas* ein konstantes Verhältnis zwischen der gebildeten Ammoniak- und Milchsäuremenge konstatieren, welches 1 : 14 entspricht. Es ist in der Muskulatur wahrscheinlich die Adenylsäure, welche die Ammoniakbildung durch Desaminierung der Adeninkomponente zu Hypoxanthin unter Entstehung von Inosinsäure bewirkt (vgl. S. 105).

Das im Urin vorkommende Ammoniak hat wahrscheinlich einen anderen Ursprung als das der Muskulatur.

Die gesteigerte Ammoniakausscheidung bei Ketonurie fand bereits früh Beachtung. Schon *Hallervorden* hielt sie für eine Neutralisierungsmassregel und meinte, die erhöhte Ammoniakmenge im Harn wäre ein Zeichen abnormer Bildung organischer Säuren im Körper. Dies führte auch zur Entdeckung der  $\beta$ -Oxybuttersäure bei gewissen Fällen von Diabetes (vgl. S. 5). *Janney* betrachtete das Urinammoniak als einen alkalisparenden Faktor bei der säuren-basenregelnden Funktion der Nieren. Bei Basenüberschuss im Organismus nimmt das Harnammoniak ab, verschwindet aber nicht völlig.

Die Frage, wo das Urinammoniak gebildet wird, ist Gegenstand eifrigen Studiums gewesen.

*Nencki*, *Paulow* und *Zaleski* fanden 1895, dass Blut aus der V. portae reichlich Ammoniak enthält, das in der Leber in Harnstoff umgewandelt wird. *Dunlop* zeigte in demselben Jahre, dass Säurezufuhr per os die Ammoniakausscheidung steigert. *Dunlop* war der Ansicht, ein Teil des Ammoniaks im Portablut werde der Harnstoffbildung in der Leber entzogen und an den Säureüberschuss gebunden. Die säuren-basenregelnde Wirkung des Ammoniaks sollte sich da in der Leber entfalten, und die Niere sollte nur bereits neutralisierte Säure ausscheiden.

Mit verbesserter Methodik haben *Denis* und *Minot*, *Nash* und *Benedict*, *Parnas*, *Henriques* u. a. die Frage der Ammoniak-

bildung aufgegriffen. Blut aus der V. portae enthält reichlich Ammoniak, das in der Darmwand bei Desaminierung von Nahrungseiweiss gebildet wird. In der Leber wird das Ammoniak irreversibel in Harnstoff umgewandelt, und zwar so vollständig, dass Blut aus der Lebervene nicht mehr Ammoniak enthält als das arterielle Blut.

*Adlersberg* und *Taubenhaus* haben in peripherem Blut nur höchstens 0,05 mg% gefunden, *Cholopoff* bis 0,02 mg%. *Henriques* u. a. bestreiten das Vorhandensein von Ammoniak im peripheren Blut. Dagegen findet sich nach *Adlersberg* und *Taubenhaus* im Blute eine ammoniakbildende lösliche Substanz, welche bei ihrem Zerfall Ammoniak liefert, bis 2 mg% in menschlichem Blut. Sie wird nicht mit dem Eiweiss ausgefällt und ist nicht mit Harnstoff identisch.

*Nash* und *Benedict*, *Parnas*, *Rabinowitsch*, *Gigon* u. a. stellen fest, dass weder bei Alkalizufuhr, welche die Ammoniakausscheidung senkt, noch bei Säurezufuhr oder etwa bei anderen Zuständen mit gesteigerter Ammoniakausscheidung eine Zunahme des Ammoniakgehalts des Blutes vorkommt. Bei erhöhter Ammoniakausscheidung ist dagegen die ammoniakbildende Substanz im Blute vermindert. *Nash* und *Benedict* haben nachgewiesen, dass das Blut aus den Nierenvenen einen höheren Ammoniakgehalt hat als das der Nierenarterie, was darauf hindeutet, dass die Ammoniakbildung in den Nieren stattfindet.

Das ausgeschiedene Ammoniak steht in einer gewissen Beziehung zur Menge des ausgeschiedenen Gesamtstickstoffs. *Hasselbalch* und *Gammeltoft* rechneten mit einem gesetzmässigen Verhältnis zwischen pH des Urins,  $H_3N$ -Stickstoff und Gesamtstickstoffausscheidung. Der prozentuale Teil des Gesamtstickstoffs, welcher dem Ammoniakstickstoff entspricht, wird als

$$\text{Ammoniakzahl} = \frac{H_3N\text{-Stickstoff} \cdot 100}{\text{Gesamtstickstoff}}$$

berechnet. Einer bestimmten Urinreaktion entspricht nach *Hasselbalch* eine bestimmte Ammoniakzahl. Die Ammoniakzahl bei pH 5,8 bezeichnet *Hasselbalch* als »reduzierte Ammoniakzahl«. In der Norm hält sich diese nach *Hasselbalch* individuell konstant, wechselt aber bei verschiedenen Personen.

*Polonowsky* und *Boulanger* fanden dagegen eine erhebliche individuelle Variationsbreite der Ammoniakzahl, welche sich mit der Ernährung und Flüssigkeitszufuhr ändert und daher keine individuell konstante Grösse sein kann. Nach *Polonowsky* und *Boulanger* sind Gesamtstickstoffausscheidung und Ammoniakausscheidung voneinander unabhängig.

TAB. 14.

Schwankungen der Ammoniakzahl während des Tages im Hunger und bei fettreicher, kohlehydratarmer Kost bei zwei Diabetikern ohne Insulin.

Fall	Zeit	U r i n				
		Menge	Gesamtstickstoff mg % A	Ammoniakstickstoff mg % B	Ammoniakzahl B · 100 A	pH
Fall 1 Hunger	2—8	320	665,4	71,4	10,7	6,1
	8—10	320	517,6	44,2	8,5	5,7
	10—12	200	378,3	23,8	6,2	5,1
	12—14	160	530,7	47,6	8,9	5,3
	14—16	300	326,1	27,2	8,3	5,7
	16—18	250	388,1	34,0	8,7	5,7
	2—6	370	797,3	74,8	9,3	5,6
	6—10	180	788,6	71,4	9,0	5,8
	10—12	220	396,3	44,2	11,1	5,8
	12—14	215	604,4	54,4	9,0	5,6
	14—16	250	400,5	40,8	10,1	5,8
	16—18	190	577,9	54,4	9,4	6,0
Fall 2 Hunger	6—8	80	808,2	51,0	6,3	5,1
	8—10	170	929,6	54,4	5,9	5,2
	10—12	160	988,1	57,8	5,8	4,9
	12—14	140	1 200,2	64,6	5,4	5,1
	14—16	125	1 232,8	81,6	6,6	5,2
	16—18	130	1 048,3	81,6	7,8	5,3
	6—8	60	766,4	122,4	16,0	5,1
	8—10	125	1 077,6	119,0	11,0	5,3
	10—12	200	569,2	91,8	16,1	5,3
	12—14	150	779,5	98,6	12,6	5,2
	14—16	180	759,4	91,8	12,1	5,5
	16—18	120	837,9	112,2	13,4	5,3

Die vorstehende Tab. 14 zeigt die Variationen der Ammoniakzahl während des Tages bei zwei Diabetikern ohne Insulin, einmal im Hunger, sodann bei einer fettreichen, kohlenhydratarmen Kost.

Aus der Tabelle geht hervor, dass die Ammoniakzahl bei Diabetes auch bei gleichbleibender Wasserstoffionenkonzentration nicht konstant ist. Es ist wahrscheinlich nicht der pH-Wert des Urins direkt, welcher für die Ammoniakzahl entscheidend ist. Die Ammoniakbildung ist eine Folge des Intermediärstoffwechsels der Nieren. Bei zunehmender Ketonurie steigt auch

TAB. 15.

Ammoniakzahl und Ammoniak- $\beta$ -Oxybuttersäurequotient bei drei Fällen von bedrohlichem Diabetes im Hunger bei Praekoma.

	Zeit	U r i n					
		Gesamt-N mg %	H <sub>3</sub> N:N mg %	Ammoniakzahl	pH	$\beta$ -Oxybuttersäure mg %	H <sub>3</sub> N:N $\beta$ -Oxybuttersäure
Evy S., 18 J. 16. I. 1940 Hunger Insulin 22 Uhr	6—10	296	50,6	17,0	5,3	425	0,12
	10—12	358	39,3	10,9	5,1	540	0,07
	12—14	215	56,0	26,0	5,0	660	0,08
	14—16	333	47,6	14,2	5,0	670	0,07
	16—18	255	58,6	23,0	5,1	675	0,09
Märta K., 33 J. 13. I. 1940 Hunger Insulin 22 Uhr	6—8	644	39,3	6,1	5,5	60	0,66
	8—10	577	36,4	6,3	5,8	110	0,33
	10—12	489	33,7	6,9	5,8	205	0,16
	12—14	430	39,0	9,1	5,7	395	0,10
	14—16	537	56,0	10,4	5,6	650	0,09
	16—18	508	56,0	11,0	5,6	505	0,11
David S., 21 J. 12. I. 1940 Hunger Insulin 16.45 und 22 Uhr	6—8	680	31,0	4,6	5,5	20	1,55
	8—10	623	31,0	5,0	5,5	255	0,12
	10—12	664	33,7	5,1	5,6	410	0,08
	12—14	627	50,4	8,0	5,7	515	0,10
	14—16	570	56,0	9,8	5,4	600	0,09
	16—18	541	56,0	10,4	5,4	680	0,08

die Ammoniakzahl; man findet immer mehr von dem ausgeschiedenen Stickstoff als Ammoniak wieder. Die nebenstehende Tab. 15 zeigt die Veränderungen der Ammoniakzahl bei zunehmender Ketonurie bei drei Fällen von schwerem Diabetes in beginnendem Praekoma.

Die Ammoniakbildung in den Nieren wird also bei normaler Nierenfunktion gesteigert, wenn die Ketonurie zunimmt.

Örström hat gefunden, dass der Glutamingehalt des Blutes insbesondere beim ketosäurenbildenden Diabetes herabgesetzt ist (S. 142). Da die Nieren reichlich Glutaminase enthalten (vgl. S. 141), kann in diesen Ammoniak in grossen Mengen abgespalten werden. Es ist daher wahrscheinlich, dass die ammoniakbildende Substanz mit dem Glutamin identisch ist.

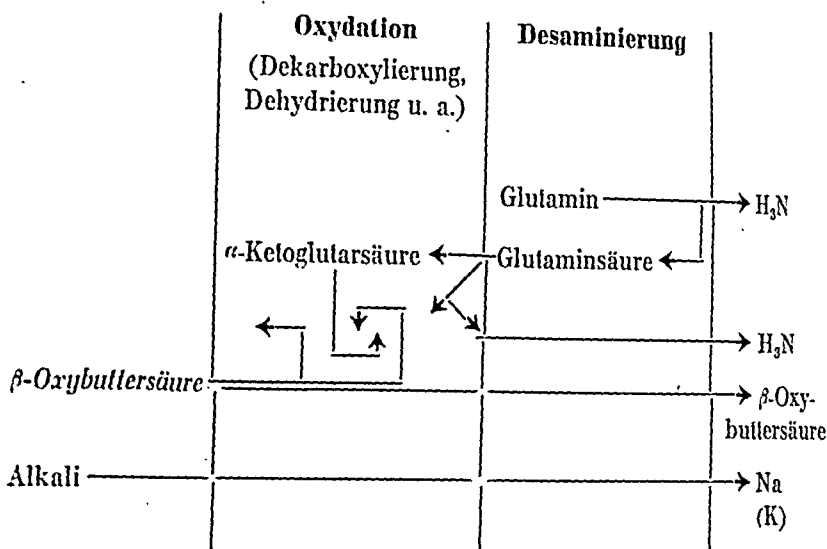
Da die Ammoniakausscheidung mit der Ketonurie zunimmt, besteht wahrscheinlich ein Zusammenhang zwischen Ammoniakbildung und Ketonkörperausscheidung. Aus Tab. 11 S. 235 wird ersichtlich, dass einem höheren hämorenalen  $\beta$ -Oxybuttersäureindex eine stärkere Ammoniakausscheidung entspricht als einem derartigen niedrigen Index. Dies kann darauf hindeuten, dass der Desaminierungsprozess in den Nieren teilweise an die Ketosäurenverbrennung in den Nieren gekoppelt ist. Ob es sich dabei um eine direkte Beziehung der beiden Vorgänge zueinander oder nur um eine indirekte Verknüpfung durch Konkurrenz um den bei der Atmung des Nierenparenchyms zur Verfügung stehenden Sauerstoff handelt, ist nicht bekannt.

Die erhöhte Ammoniakbildung bei gesteigertem oxydativem Ketonkörperabbau ist geeignet, ein Phänomen zu erklären, welches bisweilen beobachtet wird, wenn eine starke Ketonurie durch Insulinwirkung rasch verschwindet. Es tritt da nicht selten eine bald vorübergehende, sehr starke Ammoniakausscheidung auf, welche sich einige Stunden nach dem Verschwinden der Ketonurie einstellt (vgl. Abb. 48 S. 249).

Da Ammoniak die zellulären Oxydationsvorgänge anzuregen scheint (vgl. S. 107), kann das in den Nieren frei gemachte Ammoniak in bezug auf die Ketosäuren eine doppelte Aufgabe zu erfüllen haben. Einerseits kann die Geweboxydatation und damit die Ketosäureverbrennung in den Nieren durch die Ammoniakbildung gesteigert werden. Auf der anderen Seite kann

das Ammoniak als neutralisierender Faktor dienen, wodurch grössere  $\beta$ -Oxybuttersäuremengen den Organismus verlassen können, ohne dass das Nierenparenchym geschädigt wird.

Der hypothetische Verlauf bei der Verbrennung und Ausscheidung der  $\beta$ -Oxybuttersäure durch Zusammenwirken von Oxydations- und Desaminierungssystemen lässt sich schematisch in folgender Weise darstellen:



Die aus Glutaminsäure gebildete  $\alpha$ -Ketoglutaratsäure kann in dem  $\text{C}_4$ -Säurenzyklus direkt weiterumgewandelt werden und dadurch möglicherweise die oxydativen Prozesse bei Anwesenheit von Ammoniak noch mehr steigern (vgl. S. 126).

Die neutralisierende Wirkung des Ammoniaks bei der  $\beta$ -Oxybuttersäureausscheidung kann teilweise von metallischen Kationen ersetzt werden. Dabei tritt die Ammoniakbildung zurück. Wenn Alkali im Körper reichlich zur Verfügung steht, können daher grössere Mengen  $\beta$ -Oxybuttersäure ausgeschieden und der pH-Wert des Urins konstant gehalten werden, ohne dass eine komplettierende Ammoniakbildung stattfindet. Dabei wird Natrium in grösster Menge ausgeschieden.

Abb. 44 nach Lundegårdh und Möllerström veranschaulicht die alternierende Ammoniakbildung und Natriumausscheidung beim ketonkörperbildenden Diabetes. Von den in

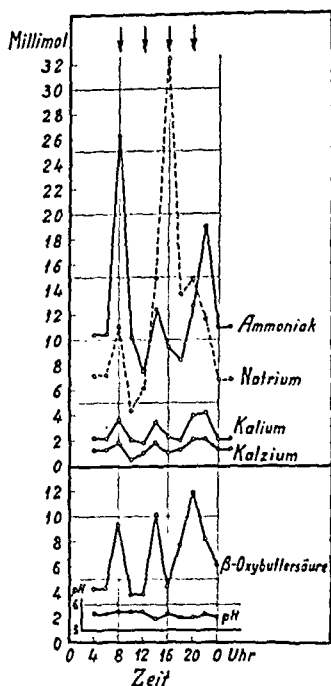


Abb. 44. Kationenausscheidung bei Ketonurie mit alternierender Natrium- und Ammoniakausscheidung bei Nahrungszufuhr ohne Insulin. Die Pfeile bezeichnen Mahlzeiten (nach Lundegårdh und Möllerström).

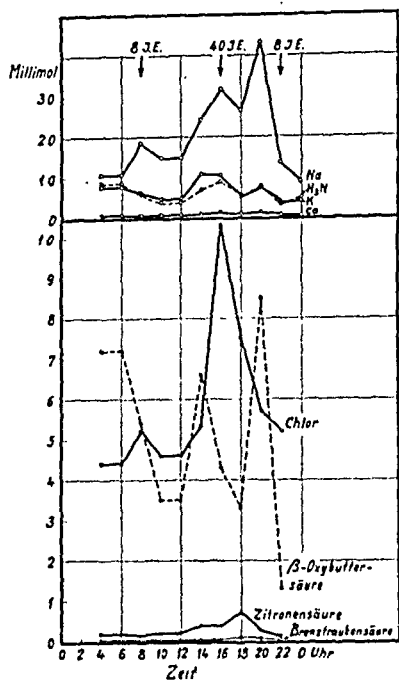


Abb. 45. Kationenausscheidung (Na, K, Ca u.  $\text{NH}_4\text{N}$ ) sowie Ausscheidung von Chlor,  $\beta$ -Oxybuttersäure, Brenztraubensäure und Zitronensäure. Chlor- und Natriumausscheidung voneinander unabhängig. Die Pfeile bezeichnen Insulin.

diesem Fall während eines Fettkosttages ohne Insulin (vgl. S. 326) auftretenden drei  $\beta$ -Oxybuttersäureausscheidungswellen entsprach der ersten und dritten eine starke Ammoniakausscheidung, der zweiten dagegen eine vermehrte Natriumausscheidung. Kalium verlässt den Körper in geringerer Menge und folgt speziell der Ketosäurenausscheidung. Kalzium dagegen wird auffallend gleichmässig, auch bei hochgradiger Azidose ohne grössere Fluktuationen, ausgeschieden. Trotz der erheblichen Variationen in der Ionenausscheidung hält sich der pH-Wert des Urins ziemlich konstant (Abb. 44). Es wird hier ersichtlich, wie Natrium und Ammoniak einander ergänzen. Phosphorsäure und anderen sauren Valenzen entspricht der Kationenüberschuss in Abb. 44 und 45.

Die Ausscheidung des Natriums erfolgt unabhängig von der



des Chlors. Es handelt sich somit nicht um eine einfache Kochsalzausscheidung. Abb. 45 nach *Lundegårdh* und *Möllerström* zeigt die Kationenausscheidung in 24 Stunden bei einem Fall von Diabetes mit Ketonurie während eines Kohlehydratkosttages mit Insulin. Neben der Ausscheidung von Natrium, Kalium, Kalzium und Ammoniak ist hier auch die von Chlor,  $\beta$ -Oxybuttersäure, Zitronensäure und Brenztraubensäure angegeben, und zwar stets in Millimol pro 2 Stunden. Der starken Chlorausscheidung um 16 Uhr entspricht in diesem Falle keine Zunahme der Natriumausscheidung; letztere tritt erst um 20 Uhr im Anschluss an eine maximale  $\beta$ -Oxybuttersäurewelle ein.

Bei sinkendem pH-Wert werden immer grössere Mengen von Ketosäuren in freier Form ausgeschieden. Nach *Henderson* kommt die  $\beta$ -Oxybuttersäure als freie Säure bei

pH 5,0 zu 33 %,

» 4,75 » 45 »,

» 4,50 » 60 »

vor.

Da die Ketosäuren ziemlich starke Säuren sind (Dissoziationskonstante bei 25°: Diazetsäure:  $2,6 \times 10^{-4}$ ,  $\beta$ -Oxybuttersäure:  $2 \times 10^{-5}$ ), können sie im Urin nicht in freier Form ausgeschieden werden, ohne dass eine Schädigung des Nierenepithels eintritt. Bei sinkendem pH-Wert wird daher die Ammoniak- $\beta$ -Oxybuttersäurebilanz des Urins für die Ausscheidungsmöglichkeiten der Ketosäuren ausschlaggebend. Ist der Ammoniak- $\beta$ -Oxybuttersäurequotient grösser als 1, so genügt die Ammoniakbildung zur Neutralisation der gesamten freien Säure. Wenn dagegen der Ammoniak- $\beta$ -Oxybuttersäurequotient kleiner als 1 ist, dann reicht das Ammoniak nicht zur Neutralisierung der ausgeschiedenen Säuren aus, und bei sinkendem pH-Wert treten mehr und mehr freie Ketosäuren im Urin auf.

Bei steigender Ketosäurenausscheidung wird auch der Verlust des Organismus an Gesamtbasen, namentlich an Natrium und Kalium, grösser. Dadurch sinkt der Kationen Gehalt des Blutes. Durch den Salzverlust nimmt auch der Wassergehalt des Blutes und der Gewebe ab. Bei dieser Austrocknung wird die Harnbildung erschwert, und es kann da eine Retention stickstoffhaltiger Produkte, eine *Austrocknungsurämie*, eintre-

ten. Wird noch dazu das Nierenparenchym durch ausgeschiedene freie Säuren geschädigt, so leidet die Nierenfunktion. Es kommt zu einer Albuminurie mit kurzen hyalinen und granulierten Zylindern, den sog. Komazylindern, im Sediment. Dann büßt die Niere immer mehr ihre Bedeutung als ein bei der Säuren-Basenregulation des Organismus wirksamer Faktor ein. Als Anzeichen der einsetzenden Niereninsuffizienz beginnt der Reststickstoff im Blute noch mehr zu steigen. Es entwickelt

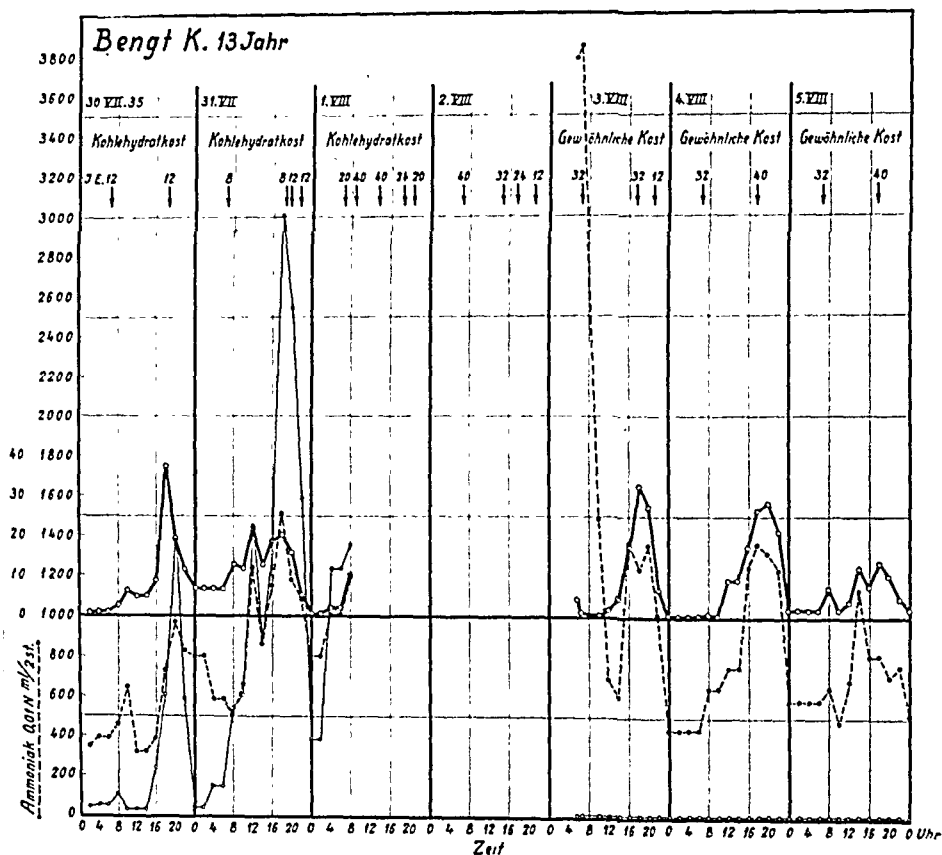


Abb. 46. Ausscheidungsdiagramm bei bedrohlichem Diabetes. Infolge von mangelhafter Insulinwirkung starke Ketosäurenbildung mit ungenügender Ammoniakausscheidung. Entwicklung eines praekomatösen Zustands mit Erbrechen und fast vollständiger Anurie. Abgesehen von einer kleineren Harnmenge am 2. VIII. morgens kam die Diurese erst am Morgen des 3. VIII. in Gang. Der Urin hatte da einen hohen Ammoniakgehalt, aber die  $\beta$ -Oxybuttersäure war durch die starke Insulinwirkung während der Anurietage spurlos verschwunden.

sich durch azidotische Nierenschädigung eine *azidotische Urämie*.

Begg fand beim Koma eine Steigerung des Harnstoffgehalts des Blutes und zugleich eine verringerte Harnstoffausscheidung, was auf eine Niereninsuffizienz hindeutet. Metzger, Kraus und Selye u. a. haben beim Koma auch histologisch nachweisbare Nierenveränderungen beobachtet.

In schweren Fällen tritt Anurie ein. Abb. 46 gibt Ausscheidungsdiagramme von einem 13jährigen Knaben mit schwerem Diabetes wieder, bei dem sich ein anurisches Praekoma entwickelte.

Nur wenn in einem derartigen Falle die Ammoniakbildung infolge der Insulinwirkung wieder zunimmt und die Ketonkörperbildung aufhört, so dass der Ammoniak- $\beta$ -Oxybuttersäurequotient steigt, kann die Nierenfunktion wieder in Gang kommen. Da verschwinden Eiweiss und Zylinder im Harn. Wäh-

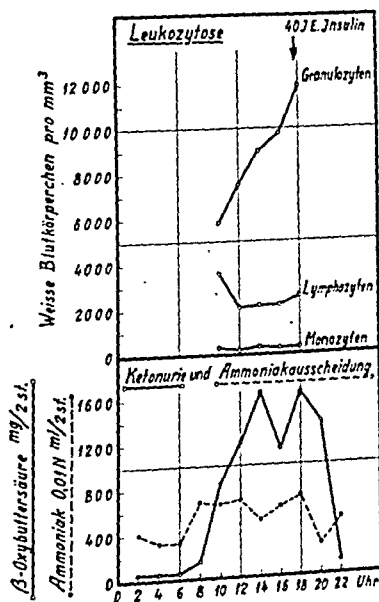


Abb. 47. Fall von bedrohlichem Diabetes. Zugleich mit der starken Ketonkörperbildung ohne gesteigerte Ammoniakausscheidung tritt ein praekomatoser Zustand ein. Dabei vermehren sich die Granulozyten im Blute, im Gegensatz zu den Lympo- und Monozyten, deren Menge unverändert ist. Mit dem Verschwinden der Ketonurie geht die Leukozytose zurück, nach Magnusson und Möllerström<sup>1)</sup>.

<sup>1</sup> Nicht veröffentlicht.

rend des Reparationsstadiums treten dabei Leukozyten in gesteigerter Menge im Urinsediment auf, nachdem die Zylinder verschwunden sind.

Schon während der Entwicklung der Azidose steigt der Leukozytengehalt des Blutes. Dabei sind es die Granulozyten, welche in erster Linie zunehmen. Abbildung 47 zeigt die Granulozytenvermehrung beim ketosäurebildenden Diabetes während eines Hungertages mit Praekoma sowie die entsprechende  $\beta$ -Oxybuttersäure-Ammoniakausscheidung.

Es ist möglich, dass die Peroxydasen der Leukozyten (vgl.

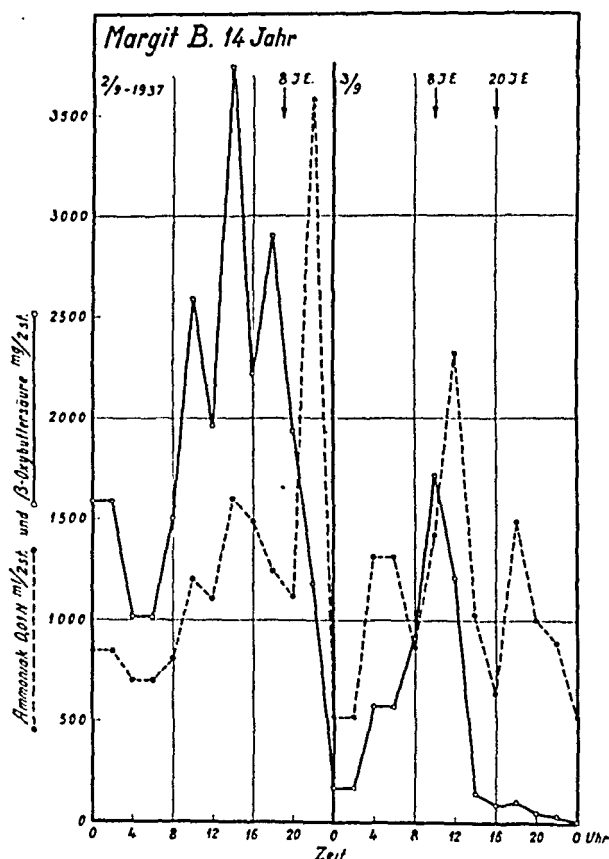


Abb. 48. Ausscheidung von  $\beta$ -Oxybuttersäure und Ammoniak bei bedrohlichem Diabetes im Praekoma. Man beachte die kräftige transitorische Ammoniakbildung bei sinkender  $\beta$ -Oxybuttersäureausscheidung nach Verabfolgung einer kleineren Insulindosis am 2. IX. abends.

S. 163) die Rolle spielen können, die oxydativen Prozesse im Organismus, namentlich im Nierenparenchym, zu steigern und damit eine erhöhte Verbrennung von Ketonkörpern und anderen störenden Zwischenprodukten zu erleichtern.

Nach Verschwinden der  $\beta$ -Oxybuttersäureausscheidung durch Insulinwirkung macht sich im Reparationsstadium oft eine *transitorische Ammoniaksteigerung* deutlich bemerkbar (vgl. Abb. 48).

## Die Reaktionsregulation des Blutes.

Ist die säuren-basenregelnde Funktion der Nieren herabgesetzt worden, so ist das Ionengleichgewicht des Blutes gestört. Dabei werden erhöhte Anforderungen an die eigene Wasserstoffionenregulationsfähigkeit des Blutes gestellt.

Die normale Reaktion des Blutes entspricht einem pH-Wert von 7,3—7,5. Messungen an nicht hämolysiertem Blut ergeben nur die Reaktion des Plasmas, etwa 7,4, unabhängig von den vorhandenen Blutkörperchen. Die Reaktion im Innern der Blutkörperchen ist normalerweise etwas saurer als im Plasma und entspricht einem pH-Wert von ungefähr 7,3. Es ist das Verhältnis zwischen basischen und sauren Valenzen, welches die Reaktion in den Blutkörperchen und im Plasma bestimmt.

TAB. 16.

**Kationenverteilung in Plasma, Blutkörperchen und Muskelzellen**  
(nach Peter und van Slyke).

	Blutplasma		Blutkörperchen		Muskelzellen	
	mg %	Milliäqu.	mg %	Milliäqu.	mg %	Milliäqu.
Natrium .....	308	144	37,9	23,1	79,9	48,0
Kalium .....	18,	4,9	313	112,2	320,1	112,5
Kalzium .....	10,0	5,4	0	0	7,5	5,2
Magnesium .....	2,0	1,7	4,8	5,5	21,2	23,9
Insgesamt		156		140,8		189,6

Blutplasma, Blutkörperchen und Körperzellen haben einen verschiedenen Ionenbestand, und das Verhältnis K : Na wechselt in den einzelnen Organen. Die nebenstehende Tab. 16 macht die Kationenverteilung in Plasma, Blutkörperchen und Muskelzellen ersichtlich, einerseits in mg% und andererseits in Milliäquivalenten pro Liter, in entsprechenden Volumina eiweissfreier wässriger Lösung angegeben.

In der Norm variiert der Gesamtbasengehalt des Blutplasmas zwischen 150 und 160 Milliäquivalenten.

Zur Aufrechterhaltung des elektrostatischen Gleichgewichts ist das Vorhandensein einer den Kationen entsprechenden Anionenmenge erforderlich. Die normale Verteilung der sauren Valenzen des Blutplasmas ist folgende:

Cl .....	103	Milliäquivalente
HCO <sub>3</sub> .....	28	»
PO <sub>4</sub> .....	2	»
Protein .....	16	»
SO <sub>4</sub> + organische Säuren ....	6	»
<hr/>		
insgesamt		155 Milliäquivalente

Der Chlorgehalt der roten Blutkörperchen beträgt normalerweise etwa  $\frac{1}{2}$  und der der Muskelzellen ungefähr  $\frac{1}{3}$  des Chlorgehalts des Blutplasmas.

Damit die Reaktion des Blutes konstant bleibe, müssen saure und basische Valenzen einander die Waage halten. Das Regulationssystem, welches dabei in Tätigkeit ist, lässt sich auf folgende Faktoren zurückführen:

1. Pufferwirkung des Hämoglobins und isohydrische Kohlensäurebindung;
2. Ionenaustausch zwischen Plasma, Blutkörperchen und Geweben;
3. Pufferwirkung des Bikarbonats;
4.       »               »       Phosphats;
5.       »               »       Plasmaeiweisses.

## Die reaktionsregelnde Wirkung des Hämoglobins

Das Hämoglobin macht ca. 34 % des Zellvolumens der roten Blutkörperchen aus. Neben seiner Funktion des Sauerstoff- und Kohlensäuretransports bei der Respiration hat das Hämoglobin bei der Säuren-Basenregulation des Blutes eine wichtige Aufgabe zu erfüllen. Die wirksamen Momente sind dabei einmal die *Pufferwirkung des Hämoglobins*, sodann die Fähigkeit desselben zur *isohydrischen Kohlensäurebindung*.

Das Hämoglobin ist ein amphoterer Eiweisskörper. Der isoelektrische Punkt des reduzierten Hämoglobins liegt bei  $\text{pH} = 6,8$ , der des Oxyhämoglobins bei  $\text{pH} = 6,65$ . Bei der normalen Reaktion der roten Blutkörperchen, welche  $\text{pH} 7,3-7,4$  entspricht, hat das Hämoglobin daher saure Eigenschaften. Es kann infolgedessen basische Valenzen binden.

Das vom Hämoglobin gebundene Alkali stellt den Hauptteil der Alkalireserve des Blutes dar. Häufen sich saure Produkte im Blute an, so werden diese an Kationen gebunden, welche aus dem Hämoglobin freigemacht werden. Dadurch sinkt die Alkalireserve des Blutes.

Nach *v. Slyke, Hastings, Heidelberger und Neill* ist das Hämoglobin bei normaler Blutalkaleszenz eine polyvalente Puffersubstanz mit mindestens fünf gepufferten sauren Valenzen. Da Alkali an alle diese Valenzen gebunden ist, besitzt das Hämoglobin eine sehr starke Pufferwirkung.

In den roten Blutkörperchen überwiegen Kaliumionen. Nach *v. Slyke, Wu und Mc Lean* wird fast die Hälfte der Kaliummenge an Hämoglobin gebunden. Der Rest der basischen Valenzen wird hauptsächlich als Chloride und Bikarbonat gebunden.

In den roten Blutkörperchen bildet sich ein Gleichgewichtszustand zwischen basischen Valenzen auf der einen und Hämoglobin,  $\text{Cl}^-$ - und  $\text{HCO}_3^-$ -Ionen auf der anderen Seite aus. Der Gleichgewichtszustand schwankt mit dem respiratorischen Gaswechsel.

Die Dissoziationskonstante des *reduzierten Hämoglobins* ist:

$$K_{\text{Hb}} = 7,5 \cdot 10^{-9}; \text{p}K_{\text{Hb}} = 8,03.$$

Durch Sauerstoffbindung wird das schwach dissoziierte Hämoglobin in *Oxyhämoglobin* umgewandelt. Die Dissoziationskonstante desselben ist:

$$K_{\text{OHHb}} = 5 \cdot 10^{-7}; \text{p}K_{\text{OHHb}} = 6,57.$$

Das Oxyhämoglobin ist daher eine stärkere Säure als das reduzierte Hämoglobin und verdrängt somit mehr Kohlensäure aus der Bikarbonatbindung als das Hämoglobin. Bei der Sauerstoffaufnahme des Hämoglobins wird gleichzeitig an Bikarbonat gebundene Kohlensäure frei gemacht. Wenn das Oxyhämoglobin wieder seinen Sauerstoff abgibt, wird das an Oxyhämoglobin gebundene Alkali frei und kann da von neuem Kohlensäure unter Bildung von Bikarbonat aufnehmen. Der normale respiratorische Gaswechsel findet ausschliesslich durch diese *isohydrische Kohlensäurebindung des Hämoglobins* statt. Die Voraussetzung dafür ist, dass beim Übergang des Hämoglobins in Oxyhämoglobin an Bikarbonat gebundenes Alkali in genügender Menge zur Verfügung steht. *Für einen ungestörten respiratorischen Gaswechsel ist eine hinreichende Alkalireserve erforderlich.*

### Das Puffersystem des Blutplasmas

Das Kohlendioxyd, welches bei Dekarboxylierungsprozessen im Organismus entsteht, geht in wässriger Lösung durch einen Hydratisierungsprozess teilweise in Kohlensäure über.



Durch Dissoziation der gebildeten Kohlensäure treten dann Bikarbonat- und Karbonationen sowie Wasserstoffionen auf.



Die Menge des gelösten Kohlendioxyds hängt teils von dem Löslichkeitskoeffizienten des Kohlendioxyds und teils von dem Druck desselben ab. Der Löslichkeitskoeffizient beträgt bei



CO<sub>2</sub> nach *v. Slyke, Sendroy, Hastings* und *Neill* für reines Wasser 0,545, für normales Plasma 0,510 (38°). Salze und Eiweisskörper im Plasma senken den Löslichkeitskoeffizient, die Anwesenheit von Lipoiden und Fett steigert dagegen die Löslichkeit des Kohlendioxyds im Plasma. Bei Lipämie kann daher die Löslichkeit des Kohlendioxyds im Plasma grösser werden als in reinem Wasser.

Der Übergang des physikalisch gelösten Kohlendioxyds in Kohlensäure nimmt eine gewisse Zeit in Anspruch. Der Hydratisierungsprozess wird beim biologischen Gaswechsel durch die Wirkung eines in den Geweben vorkommenden spezifischen Ferments, der von *Brinkmann, Margaria* und *Roughton* entdeckten *Carboanhydrase*, in hohem Grade beschleunigt. Die Carboanhydrase ist nach *Keilin* und *Mann* ein Zinkprotein, welches etwa 0,3 % Zn enthält. Sie findet sich in den roten Blutkörperchen. Durch die Wirkung der Carboanhydrase wird die Hydratisierungszeit für das gelöste Kohlendioxyd dermassen reduziert, dass sich schon beim Gaswechsel zwischen Blut und Geweben dissoziierbare Kohlensäure bildet.

Bei Anwesenheit von verfügbarem Alkali im Blutplasma wird die Kohlensäure zum Teil als Bikarbonat gebunden. Im arteriellen Blute entspricht der Kohlensäuredruck normalerweise 35—45 mm Hg, im venösen ist er in der Ruhe ca. 5—7 mm Hg niedriger.

Bei einem CO<sub>2</sub>-Druck von 40 mm Hg sind in der Norm etwa 43—56 Volumenprozent CO<sub>2</sub> als Bikarbonat im Blute gebunden.

Zwischen der Wasserstoffionenkonzentration, dem Kohlensäuregehalt und dem Bikarbonatgehalt des Blutes herrscht eine gesetzmässige Beziehung. Diese lässt sich nach dem Gesetz der Massenwirkung berechnen.

Nach diesem ist

$$\frac{[H^+][HCO_3^-]}{[H_2CO_3]} = K.$$

Die Wasserstoffionenkonzentration wird daher

$$[H^+] = \frac{K \cdot [H_2CO_3]}{[HCO_3^-]}$$

Von dem Bikarbonat ist ein Teil undissoziiert, und die freien Bikarbonationen werden von der Dissoziationskonstante des Bikarbonats  $C$  bestimmt. Werden die Konstanten zu einer gemeinsamen Konstante vereinigt, welche der Dissoziationskonstante der Kohlensäure  $K'$  entspricht, so erhält man

$$[H^+] = K' \frac{[H_2CO_3]}{[HCO_3^-]}, \text{ oder}$$

$$pH = pK' + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

Mit steigendem  $CO_2$ -Druck nimmt der Kohlensäuregehalt zu, und damit sinkt der pH-Wert.

Die hier folgende Tabelle zeigt nach *Hastings* und *Sendroy*, in welcher Form das Kohlendioxyd bei wechselndem pH-Wert gebunden ist.

TAB. 17.

Bedeutung der Wasserstoffionenkonzentration für die Kohlensäurebindung (nach *Hastings* und *Sendroy*).

pH	$H_2CO_3$ %	$BHCO_3$ %	$B_2CO_3$ %
7,0	11,0	88,8	0,16
7,4	4,8	94,8	0,4
7,8	2,0	97,0	1,0

Bei normaler Blutplasmareaktion, welche einem pH-Wert von 7,4 entspricht, ist mithin der grösste Teil der Kohlensäure des Plasmas als Bikarbonat gebunden.

Bei Anwesenheit anderer Säuren als Kohlensäure können diese zu einem kleinen Teil durch das Puffersystem



im Blutplasma ohne Änderung der Reaktion des Blutes gebunden werden. Dabei wird  $CO_2$  frei gemacht, welches mit der Respirationsluft abgeht, wobei der Bikarbonatgehalt des Blutes geringer wird. Durch die Ausscheidung des Kohlendioxyds

in der Respirationsluft wird Alkali verfügbar, welches andere Säuren binden kann.

### Die Chlorionenwanderung

Schon die isohydrische Kohlensäurebindung ist mit einem Ionenaustausch zwischen Blutkörperchen und Plasma verbunden.

Da die Blutkörperchen von einer semipermeablen Membran umgeben werden und sich in einem eiweisshaltigen Medium befinden, entwickelt sich zwischen den monovalenten Ionen des Plasmas und der Blutkörperchen ein *Donnan-Gleichgewicht*.

Wenn der  $\text{CO}_2$ -Gehalt der Blutkörperchen beim respiratorischen Gaswechsel zunimmt, steigt auch der Gehalt derselben an  $\text{HCO}_3$ -Ionen. Diese wandern teilweise in das Plasma aus. Zur Erhaltung des Ionengleichgewichts müssen da  $\text{Cl}^-$ -Ionen aus dem Plasma in die roten Blutkörperchen einwandern. Hierbei wird Alkali im Plasma frei gemacht, hauptsächlich  $\text{Na}^+$ -Ionen, welche mit den ausgewanderten  $\text{HCO}_3$ -Ionen  $\text{NaHCO}_3$  bilden.

*Zuntz* und *Loewi* beobachteten, dass die titrierbare Alkalimenge im Plasma stieg, wenn  $\text{CO}_2$  durch das Blut geleitet wurde. Gleichzeitig nahm der Chlorgehalt des Plasmas ab.

Bei normaler Ruheatmung wird das gesamte gebildete  $\text{CO}_2$  isohydrisch ohne Belastung des Puffersystems des Blutplasmas gebunden.

Treten stärkere Säuren als Kohlensäure auf, z. B. Milchsäure bei Muskelarbeit oder Ketosäuren beim Diabetes, so wird in erster Linie das Bikarbonatsystem des Blutplasmas beeinflusst und Kohlensäure frei gemacht, welche den Körper bei der Atmung verlässt. Dabei findet ein Ionenaustausch zwischen Plasma und Blutkörperchen statt, und die Alkalireserve des Blutes sinkt. Dabei wandern auch Chlorionen aus den Blutkörperchen in das Plasma, und infolgedessen sinkt der Chlorgehalt der Blutkörperchen (Abb. 49). Der Chlorüberschuss im Plasma wird mit dem Urin ausgeschieden, dessen

Chlorgehalt steigt (Abb. 49 b). Aus diesem Grunde kann der Chlorgehalt des Blutplasmas trotz der Chlorionenwanderung relativ konstant sein.

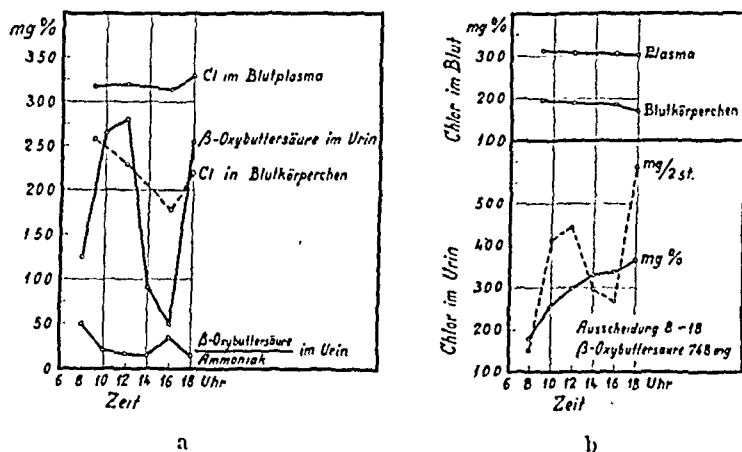


Abb. 49. Chlorgehalt in Blutkörperchen und Plasma bei zwei Fällen von ketonkörperbildendem Diabetes während eines Hungertages ohne Insulin.  
 a Chlorionenwanderung bei ungenügender  $\beta$ -Oxybuttersäureausscheidung.  
 b Gesteigerte Chlorausscheidung im Urin bei beginnender Chlorionenwanderung.

Die Chlorionenwanderung wird damit zu einem für die Säuren-Basenregulation des Blutes bedeutungsvollen Faktor.

### Die Alkalireserve

Der Bikarbonatgehalt des Blutes bildet einen Massstab für die verfügbare reaktionsregelnde Alkalimenge desselben. V. Slyke hat daher den klinischen Begriff »Alkalireserve« als Ausdruck für diesen Bikarbonatgehalt eingeführt.

Die Alkalireserve wird durch Messung derjenigen Menge Kohlendioxyd bestimmt, welche das Blut bei einem Druck von 40 mm Hg, der dem normalen arteriellen Kohlensäuredruck entspricht, zu binden vermag. Nach Odin entspricht die Kohlensäurekapazität bei Gesunden 45—50 Volumenprozent.

Bei anaerober Entnahme von Blutproben, wobei das Entweichen von Kohlendioxyd aus dem Blute verhindert wird, lässt sich nach Kirk die Alkalireserve am einfachsten durch

direkte volumetrische oder manometrische Messung derjenigen Kohlensäuremenge, welche nach Zusatz von Milchsäure im Vakuum aus dem Blutplasma frei wird, nach van Slyke bestimmen. Dabei erhält man einmal das im Plasma gelöste freie Kohlendioxyd, sodann das in Form von Bikarbonat gebundene. Ersteres tritt gegenüber dem letzteren ganz zurück. Normalerweise schwankt nach Kirk die frei gemachte Kohlensäuremenge zwischen 50 und 75 Volumenprozent, wovon ca. 5 % aus freier Kohlensäure und der Rest aus als Bikarbonat gebundener, ca. 20—30 Millimol entsprechend, bestehen.

Bei Anhäufung von Säuren im Blute sinkt die bikarbonatgebundene Kohlensäuremenge bei leichteren Graden von Azidose auf 35—50 Volumenprozent  $\text{CO}_2$ , was 15—21 Millimol entspricht. Bei Azidose höheren Grades nimmt der Kohlensäuregehalt bis zu 20—35 Volumenprozent ab.

Im Blutplasma ist neben dem Bikarbonatsystem noch das Plasmaciweiss sowie in geringerem Grade auch das Plasmaphosphat als Puffer wirksam. Dazu kommt ferner die Mitwirkung der roten Blutkörperchen und des Hämoglobins als bei der Reaktionsregulierung des Blutes beteiligte Faktoren.

Bei leichteren Graden von Azidose mit mässiger Anhäufung von Säuren liegt eine *kompensierte Azidose* mit *Hypokapnie* vor, die einem normalen pH-Wert im Blute bei jedoch verminderter Alkalireserve entspricht. Gegenüber der reaktionsstabilisierenden Wirkung des Hämoglobins und der Chlorionenwanderung tritt die Pufferwirkung des Blutplasmas hier zurück. Bei weiterer Anhäufung von Säuren sinkt daher der pH-Wert des Blutes rasch. Es tritt eine *dekompensierte* oder *echte Azidose* ein, mit gesenktem pH-Wert und mit *Hyperhydrie*, und zwar infolge der erhöhten Wasserstoffionenkonzentration.

Ist die Alkalireserve so stark gesunken, dass sie nur 20—25 Volumenprozent gebundenen  $\text{CO}_2$  bei einem Druck von 40 mm Hg, oder weniger als 10 Millimol Bikarbonat, entspricht, so droht ein azidotisches Koma.

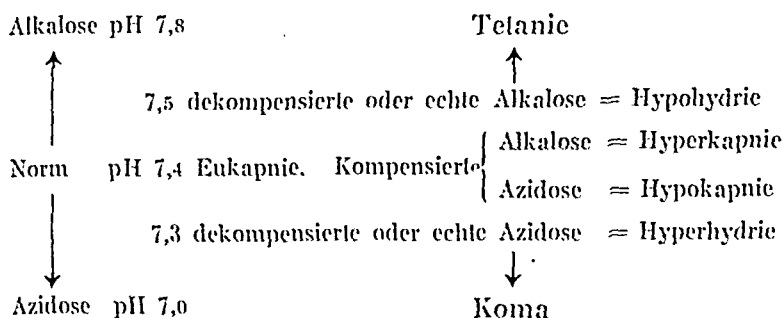
Wenn dagegen der Alkaligehalt des Blutes zunimmt, dann tritt zunächst eine *kompensierte Alkalose* mit *Hyperkapnie* ein, welche einem normalen pH-Wert, aber mit erhöhter Al-

kalireserve, entspricht. Steigt der Alkaligehalt noch weiter, so entwickelt sich infolge der verminderten Wasserstoffionenkonzentration eine *dekompensierte Alkalose* mit Hypohydrie. Dabei steigt der pH-Wert des Blutes.

Bei normalem pH-Wert des Blutes mit normaler Alkalireserve sprechen wir von einer *Eukapnie*.

Eine hochgradige Alkalose führt zum klinischen Bilde der *Tetanie*, eine hochgradige Azidose dagegen zum *Koma*.

Bei Veränderungen des pH-Werts des Blutes entwickeln sich also folgende Zustände:



### Der alveoläre Kohlensäuredruck

Der Kohlensäuregehalt des arteriellen Blutes befindet sich nach *Krogh* im Gleichgewicht mit dem der Alveolarluft. Ist die Kohlensäurebindungsfähigkeit des Blutes herabgesetzt, so nimmt auch die Kohlensäureabgabe an die Alveolarluft ab. Bei fallender Alkalireserve sinkt daher auch der Kohlensäuregehalt der Alveolarluft.

Nach *Haldane* und *Priestley* entspricht die Respirationsluft, welche man am Schluss einer forcierten Expiration erhält, der alveolären Luft. Der Kohlensäuregehalt derselben befindet sich in der Ruhe und unter normalen Verhältnissen mit dem des arteriellen Blutes im Gleichgewicht.

Die Veränderungen des Kohlensäuregehalts der Alveolarluft laufen träger ab als die Schwankungen des Kohlensäuregehalts und der Alkalireserve des Blutes. Der alveoläre Kohlensäuregehalt sinkt daher bei beginnender Azidose langsamer als die

Alkalireserve und steigt beim Verschwinden der Azidose auch langsamer als der Bikarbonatgehalt des Blutes.

Bei Anhäufung organischer Säuren mit Verminderung der Alkalireserve und Erschwerung des respiratorischen Gaswechsels kommt es zu einer Reizung des Atmungszentrums mit verstärkter Respiration. Hierdurch wird der respiratorische Gaswechsel bis zu einem gewissen Grade erleichtert. Bei einer Azidose ist nicht nur der Kohlensäuretransport durch die ungenügende Alkalireserve erschwert, sondern auch die Sauerstoffbindung des Hämoglobins, und dabei nimmt die Oxyhämoglobinbildung bei sinkendem pH-Wert ab. *Henderson und Bock* haben gefunden, dass bei einem Sauerstoffdruck von 30 mm Hg bei pH 7,64 73 % des Hämoglobins in Oxyhämoglobin umgewandelt werden, bei pH 7,24 aber nur 45 %. *Eine Azidose stört daher bereits die Sauerstoffaufnahme des Hämoglobins und erschwert dadurch die Oxydationsprozesse im Organismus.*

Die Veränderung der Respiration tritt ein, wenn die Kohlensäurebindungsfähigkeit des Blutes 30—40 Volumenprozent CO<sub>2</sub> bei einem Druck von 40 mm Hg entspricht. Nimmt die Hypokapnie zu, so entwickelt sich das Symptom der *Kussmaulschen* Atmung.

Wenn die regulatorische Wirkung des Atmungsmechanismus nicht länger zur Kompensation des durch die Senkung der Alkalireserve erschwerten Gaswechsels ausreicht, beginnt der pH-Wert des Blutes zu sinken.

*Endres* beobachtete bei einem alveolären Kohlensäuredruck von 20 mm Hg einen pH-Wert des Blutes, der 7,21 entsprach. Bei einem Koma diabeticum betrug der pH-Wert des Blutes 6,95, bei entsprechendem Kohlensäuredruck in der Alveolarluft von nur 11,3 mm Hg.

*Straub* fand bei einem Fall von diabetischem Koma einen alveolären Kohlensäuredruck von rund 14 mm Hg; der entsprechende pH-Wert des Blutes war 7,07.

*Elmer* und *Scheps* haben versucht, die Alkalireserve aus der alveolären Kohlensäuredruck in mm Hg durch einen empirischen Faktor zu berechnen:

Alkalireserve =  $1,5 \times$  alveolärer CO<sub>2</sub>-Druck in mm Hg.

In ca. 70 % sämtlicher Fälle stimmt diese empirische Formel, in den übrigen besteht eine grössere oder geringere Diskrepanz.

Die Bestimmung des Kohlensäuregehalts der Alveolarluft vermag daher Alkalireservebestimmungen nicht zu ersetzen, ist aber für rasche Orientierung wertvoll, wenn es sich darum handelt, eine progrediente Azidose bei einem drohenden Koma zu verfolgen. Eine Voraussetzung ist natürlich, dass die Kohlensäurebestimmung wirklich in Alveolarluft vorgenommen wird, welche sich mit dem arteriellen Blute im Gleichgewicht befindet. Diese Schwierigkeit macht die Untersuchung der Alveolarluft unsicherer als die direkte Bestimmung der Alkalireserve nach *v. Slyke*.



## VIII. ABSCHNITT

# Die Bedeutung des endogenen Rhythmus für die Analyse des diabetischen Zustands.

Eine Störung im Zwischenstoffwechsel mit Herabsetzung oder Veränderung der Glykogenbildung kann zu einem Diabetes mellitus führen. Die Störung kann durch einen Mangel an für den normalen Verlauf der Prozesse erforderlichen Biokatalysatoren oder durch eine Verschiebung der neurohormonalen Blutzuckerregulation von organischer oder funktioneller Natur zustande kommen. Man kann daher die diabetische Stoffwechselstörung grundsätzlich in zwei grosse Hauptgruppen einteilen (vgl. S. 198):

1. Mangeldiabetes,
2. Regulationsdiabetes.

Diese Diabetesformen gehen ineinander über. *Es handelt sich daher hier nicht um ein klinisches Einteilungsprinzip sondern um einen grundlegenden Gesichtspunkt bei der Erörterung des Diabetesproblems.*

## Mangeldiabetes

Bei biologisch unzulänglicher Ernährung fehlen in der Kosakzessorische Bestandteile, welche in Form der Vitamine zu einem normalen Stoffwechsel nötig sind. Bei Mangelzuständen verschiedener Art kann es zu Störungen auch im Kohlehydratumsatz kommen, wodurch ein bereits vorhandener Diabetes verschlimmert oder evtl. ein Diabetes ausgelöst wird. Man

muss auch mit der Möglichkeit rechnen, dass der Vitaminumsatz im Zusammenhang mit der allgemeinen Stoffwechselstörung beim Diabetes beeinträchtigt sein kann, und dass dies eine ungenügende Vitaminwirkung im Organismus zur Folge hat. Störungen im Phosphorylierungsprozess können z. B. wahrscheinlich auch hinsichtlich derjenigen Biokatalysatoren eintreten, welche zu ihrer Funktion im Stoffwechsel eine Mitwirkung von Phosphorsäure erfordern.

## Vitamine und Diabetes

### *Vitamin A*

Der Umsatz von Vitamin A scheint bei gewissen Fällen von Diabetes gestört zu sein. *Ralli* fand bei Diabetikern eine Steigerung des Karotingehalts im Blut. Seiner Ansicht nach beruht dies auf einer mangelhaften Fähigkeit der Leber, Karotin in Vitamin A umzuwandeln.

*Brazer* und *Curtis* haben durch Untersuchung der Adaptierung des Auges an Dunkelheit beim Diabetes eine herabgesetzte A-Vitaminwirkung im Organismus konstatiert. *Murrit* und Mitarbeiter fanden jedoch bei direkter Bestimmung des A-Vitamingehalts im Blut bei 16 Diabetikern keine sichere Senkung, wohl aber einen erhöhten Karotingehalt des Serums. Die Karotinämie beim Diabetes beruht daher wahrscheinlich nicht auf einer mangelhaften Umwandlung von Karotin in Vitamin A. Es ist möglich, dass die Gewebe das im Blut vorhandene Vitamin A nicht ausnützen können: hierüber ist indessen nichts bekannt.

Das Eingreifen des Vitamin A in den intermediären Stoffwechselprozess ist ganz unbekannt, ebenso die Bedeutung desselben beim Diabetes. Vielleicht hängt die beim Diabetes zuweilen vorkommende Verminderung der Widerstandsfähigkeit gegen Infektionen mit Störungen des A-Vitaminumsatzes im Organismus zusammen, aber hierüber ist nichts bekannt.

### *Vitamin B*

Der B-Vitaminskomplex ist für den Aufbau gewisser Atmungsfermente im Organismus erforderlich. Namentlich sind

diejenigen Fermentsysteme, welche bei den biologischen Dehydrierungs- und Dekarboxylierungsvorgängen in den lebenden Zellen ihre Wirkung entfalten, von der Anwesenheit von Vitamin B abhängig (vgl. V. Abschnitt). B-Vitaminmangel bewirkt daher eine Verschlechterung des Kohlehydratumsatzes sowie auch Störungen in der Glykogenbildung (s. S. 50).

Im Gegensatz zu niederen Organismen kann der Mensch Vitamin B<sub>1</sub> wahrscheinlich nicht selbst bilden. Durch die Symbiose mit *Bact. coli*, welches Aneurin zu bilden imstande ist, kann der Mensch bei normaler Darmflora und -funktion die notwendige B<sub>1</sub>-Vitaminmenge erhalten, wenn die Nahrung nicht genug davon enthält. Bei Störungen der Darmfunktion kann die enterogene Aneurinbildung abnehmen, wobei sich ein Mangelzustand entwickeln kann, der eine Rückwirkung auf den Kohlehydratstoffwechsel ausübt.

Das Aneurin muss, um seine Wirkung entfalten zu können, an Pyrophosphorsäure gekoppelt sein (vgl. S. 173). Unterbleibt dieser Phosphorylierungsprozess, so fällt die Wirkung des Vitamins B<sub>1</sub> weg, auch wenn dasselbe in reichlicher Menge zugeführt wird. Da beim Diabetes Störungen in den Phosphorylierungsvorgängen des Organismus auftreten können, ist es möglich, dass auch das Vitamin B<sub>1</sub> bei dieser Krankheit nicht immer in wirksame Cokarboxylase übergeführt wird; dadurch kommt es zu einer beträchtlichen Verschiebung im normalen Kohlehydratstoffwechsel, durch die der diabetische Zustand trotz des Vorhandenseins von Aneurin noch mehr verschlimmert wird.

### *Vitamin C*

Beim Skorbut nimmt die Glykogenbildung in der Leber ab (s. S. 51). Bei C-Vitaminmangel stellen sich infolgedessen Störungen im Kohlehydratstoffwechsel ein, welche zur Azidose und Glykosurie führen können.

Beim Diabetes kommt nicht selten eine C-Hypovitaminose im Zusammenhang mit einer einseitigen, vitaminarmen Kost vor. Wird da Ascorbinsäure in reichlicher Menge zugeführt, so bessert sich auch der diabetische Zustand. Söderling und Hamne u. a. haben mit Skorbut kombinierte Diabetesfälle beschrieben.



behandlung mit Insulin. Bei der Entlassung wurde nicht Insulin, sondern Synthalin verordnet, welches Pat. indessen nicht einnehmen konnte, da er auf dasselbe mit Temperatursteigerung reagierte. Die Insulinbehandlung musste daher nach kurzer Zeit wiederaufgenommen werden.

Bei Beobachtung im März 1941 waren bis auf Ausfall des rechten Achillessehnenreflexes und etwas ungleichmässige Pupillenreflexe keinerlei Nervensymptome nachweisbar. Das Ekg ergab verlängerte Überleitungszeit (0,20 Sek.), Herz sonst o. B. Blut: Hb. 91 %, rote Blk. 4,16 Mill., weisse 8600. SR 14—39. Übrige Organe o. B. Der Diabetes-typus wird aus dem hier folgenden Ausscheidungsdiagramm ersichtlich (Abb. 50).

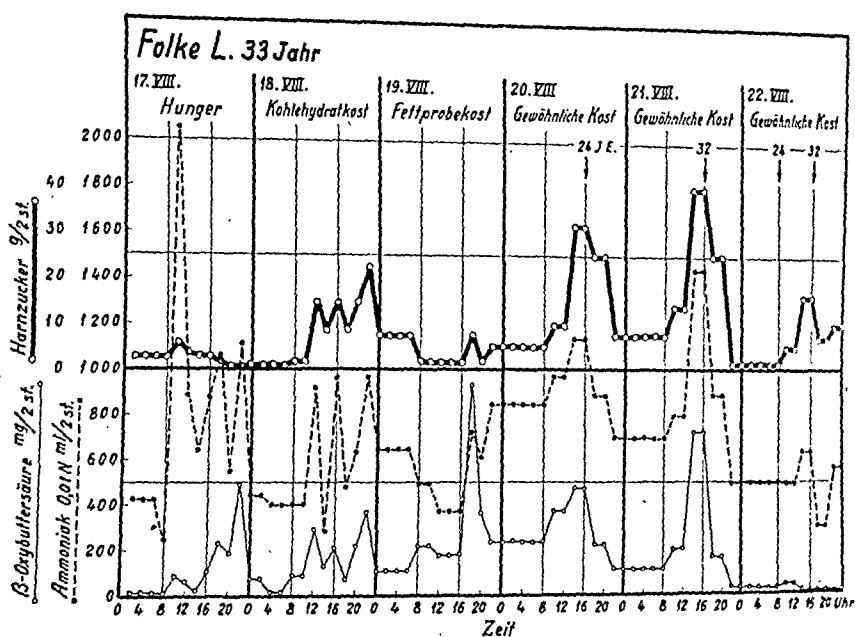


Abb. 50. Ausscheidungsdiagramm bei ketosäurenbildendem Diabetes, der sich nach einer Kohlenoxydvergiftung schleichend entwickelte.

Andere Vergiftungen können toxische Wirkungen verursachen, welche ihrerseits einen Diabetes auslösen können. Ein Beispiel hierfür ist der folgende Fall von subakuter Zinkvergiftung:

Fall 5. Karl M., geb. 1910, Metallgiesser.

Keine erbliche Veranlagung für Diabetes. Früher gesund. Arbeitete seit Mai 1936 mit Zinkguss. Hatte bei dieser Arbeit wiederholt Anfälle von sog. Giessfieber in der Nacht. Die Anfälle begannen gewöhnlich abends mit Übelkeit, Erbrechen und leichter Temperatursteigerung bis 38°. Sie traten nach ein- bis zweitägiger Beschäftigung

mit Zinkguss auf, wobei Pat. die Dämpfe des geschmolzenen Metalls einatmete. Nachts Schüttelfrost und leichte Krämpfe, welche nach etwa einer Stunde aufhörten. Am nächsten Tage fühlte sich Pat. etwas matt und litt gewöhnlich an leichten Kopfschmerzen, war aber sonst munter und arbeitsfähig. Die Müdigkeit nahm jedoch nach jedem Anfall von Giessfieber zu. Ende März 1937 katarrhalische Infektion mit Temperatursteigerung auf  $38,7^{\circ}$ , die nach einigen Tagen abklang, worauf Pat. seine Arbeit mit Zinkguss wiederaufnahm. Nach weiteren 1 Tagen ein neuer Anfall von Giessfieber. Am nächsten Tage setzte ganz plötzlich ein starkes Durstgefühl ein (Pat. trank ca. 6 Liter in 21 Stunden), ferner Jucken an den Füßen. Nach einem weiteren Tage wurde Diabetes konstatiert, dreiwöchige Krankenhausbehandlung. Fett-Gemüsekost mit 75 g Brot ohne Insulin. Im November 1937 leichte Sehstörungen.

Bei der Beobachtung 2. III.—17. III. 1938 lag ein stark säurebildender Diabetes sowie eine beginnende Kataraktbildung vor.

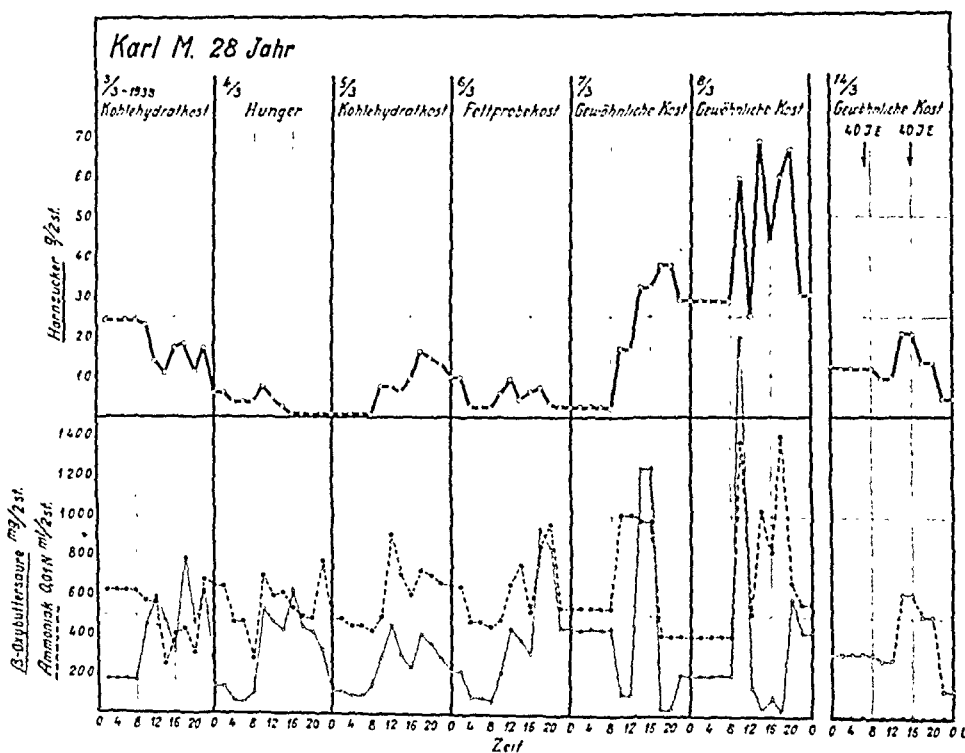


Abb. 51. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes, der im Anschluss an eine akute Vergiftung mit Zinkdämpfen auftrat. Günstiger Einfluss von Insulin. Man beachte den Verlauf der  $\beta$ -Oxybuttersäureausscheidung während des Hungertages und das Verschwinden derselben nach Einleitung der Insulinbehandlung.

Das Ausscheidungsdiagramm bei diesem Fall ist in Abb. 51 wiedergegeben. Es zeigt das Verschwinden der Ketonurie nach der Verabreichung von Insulin.

Über die Bedeutung der Zinkionen für den Zwischenstoffwechsel vgl. S. 123 u. 127.

Ein typischer Diabetes auslösender Vergiftungszustand ist die *Phloridzinvergiftung*. Hier ist der Phosphorylierungsprozess in der Niere gestört, wobei die normale Rückresorption der Glykose in den Nierenkanälchen verhindert wird. Es entsteht da ein Diabetes vom renalen Typus.

Bei *Leberschädigungen* mit mangelhafter Funktion des Leberparenchyms und Störungen der Glykogenbildung entwickelt

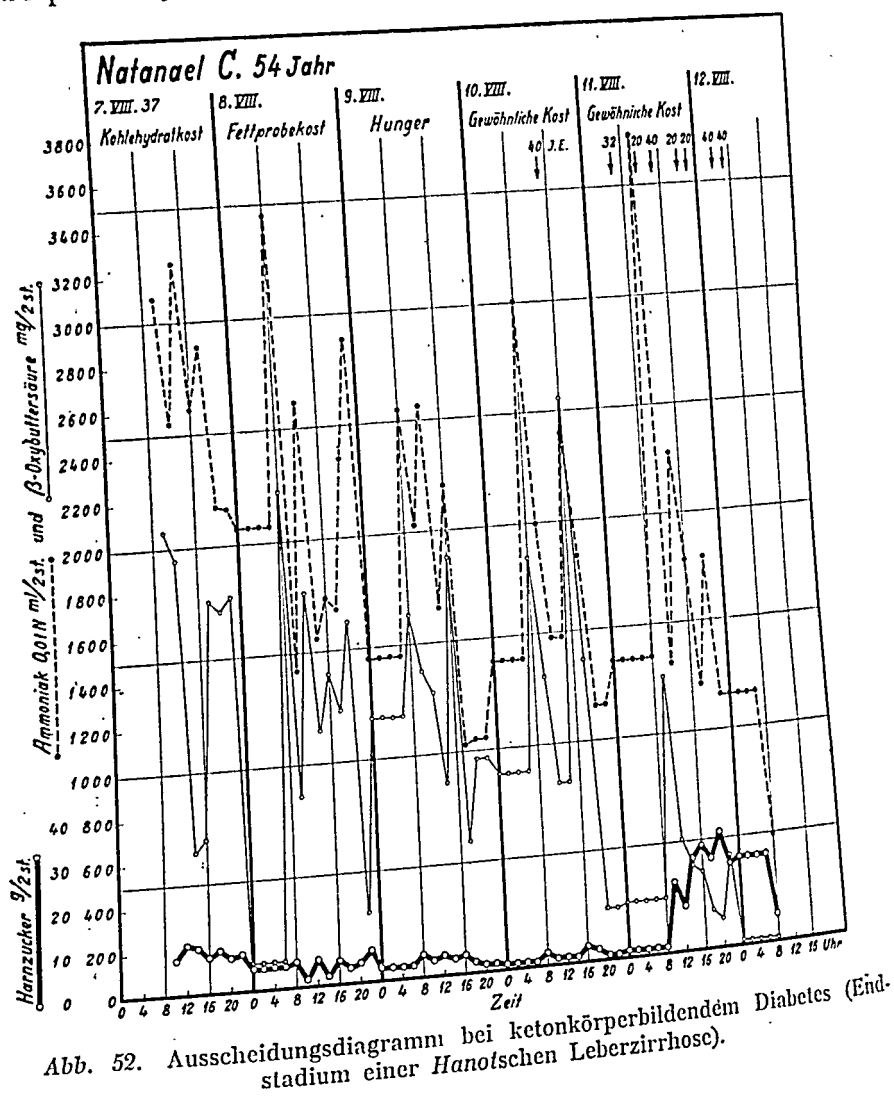


Abb. 52. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes (Endstadium einer Hanotschen Leberzirrhose).

sich nicht selten ein Diabetes. Dieser ist daher bei Erkrankungen der Gallenwege oder anderen Leberaffektionen nichts Ungewöhnliches. Der folgende Fall stellt ein Beispiel für Diabetes bei einer hochgradigen Leberzirrhose vom *Hanotschen* Typus dar:

*Fall 6.* Nathanael C., geb. 1883.

Ein Bruder leidet an Diabetes. Im Frühjahr 1936 zunehmender Durst und Müdigkeit. Der Diabetes wurde im Juni 1936 konstatiert. Pat. hielt eine sehr strenge Diät, nahm in einem Jahre 35 kg ab, fühlte sich aber bis Juni 1937 ziemlich wohl. Da rasche Verschlimmerung mit zunehmendem Schwächegefühl und gesteigertem Durst.

Bei der Beobachtung im August 1937 erhebliche Lebervergrößerung, aber kein nachweisbarer Aszites. Kein Ikterus. Allgemeinzustand ganz elend, Pat. ist hochgradig abgemagert. Herz o. B. Der Diabetes-typus geht aus dem nebenstehenden Diagramm Abb. 52 hervor.

Die Insulinbehandlung wurde eingeleitet, wobei die Azidose rasch aufhörte. Die Zuckerausscheidung, nahm nach Einleitung der Insulinbehandlung vorübergehend zu, verschwand später. Der Zustand verschlimmerte sich trotz dessen ununterbrochen, und Pat. verstarb nach einigen Tagen in einem komatösen Zustand. Bei der Autopsie fand man eine hochgradige Leberzirrhose mit starker bindegewebiger Umwandlung und nur geringfügigen Resten von Leberparenchym.

### Mangelzustände mit inneren Ursachen

Bei organischen Schädigungen hormonproduzierender Zellsysteme kann ein Mangel an einem zur normalen Kohlehydratumsatzung notwendigen Hormon entstehen. Ein solcher Mangel kann auch durch funktionelle Störungen zustande kommen. Ein Regulationsdiabetes kann demnach bei schwereren Formen in einen endogenen Mangeldiabetes übergehen. Von spezieller Bedeutung für den Kohlehydratstoffwechsel ist hierbei der Mangel an Insulin, welcher zur Entstehung eines Diabetes führt. Weil die Insulinproduktion unter nervösen Kontrolle seitens des Vagus und Sympathicussystems steht (vgl. S. 197), kann eine veränderte Hormonproduktion durch abnorme Reizimpulse zustande kommen, welche die Hormon erzeugenden Organe erreichen. Eine mangelnde Insulinbildung kann auch durch eine Atrophie des Drüsengewebes infolge von ungenügender adenotroper Hormonwirkung entstehen.



Zu den endogenen Mangelzuständen kann man auch eine *unzulängliche Sauerstoffzufuhr* zu den Geweben und Organen durch Veränderung des Sauerstofftransports, der Zirkulation, oder durch Erschwerung des Gasaustausches zwischen Blut und Geweben bei Schädigungen des Herzens und Gefäßsystems rechnen. Durch den Sauerstoffmangel erfolgt eine Herabsetzung der biologischen Atmungsfunktionen mit Störungen des Kohlehydratumsatzes in den lebenden Zellen. Dabei kann ein Asphyxiadiabetes entstehen, und zwar auf dieselbe Weise, wie bei Schädigung der sauerstoffübertragenden Fermentsysteme.

Bei Versagen der Herzkraft mit daniederliegender Glykogenbildung in der Herzmuskulatur kann die Blutzuckersteigerung den Ernährungszustand des Herzens bis zu einem gewissen Grade verbessern. Die Hyperglykämie kann da als eine Schutzmassnahme des Organismus gegen die Herzschwäche betrachtet werden — eine natürliche Glykosetherapie für den geschwächten Herzmuskel. *Eine schematische Diabetesbehandlung, ohne Rücksicht auf den Zustand des Herzens, kann zu einer beträchtlichen Verschlimmerung des Gesamtzustands führen.* Der folgende Fall ist ein Beispiel hierfür:

Fall 7. Anders J., geb. 1877.

Keine erbliche Veranlagung für Diabetes. Der Diabetes wurde 1923 bei Gelegenheit einer Operation wegen gangränöser Appendizitis konstatiert. Pat. hielt in den folgenden Jahren eine mässige Diät und nahm 10 kg ab (von 96 auf 86 kg). War die ganze Zeit munter, keine Herzbeschwerden, doch zeitweise leichtes Durstgefühl. Im Februar 1936 etwas Herzbeschwerden, zunehmende Müdigkeit, schlechterer Appetit und zuweilen Übelkeit. Pat. konsultierte einen Arzt, welcher einen Herzfehler feststellte und Digitalis verordnete. Nach einigen Tagen begann Erbrechen, welches auf den Diabetes zurückgeführt wurde. Es wurde eine energische Insulinbehandlung eingeleitet (3mal tägl. 48 I. E.).

In unmittelbarem Anschluss an die Insulinbehandlung starke Verschlimmerung des Zustandes mit zunehmenden Ödemen, besonders an den Beinen, präkordialen Schmerzen und Atemnot. Pat. konnte infolge der Atembeschwerden schlecht liegen. Die Insulindosis wurde nach und nach auf 2mal tägl. 32 I. E. herabgesetzt.

Am 2. III. 1936 wies Pat. erhebliche kardiale Ödeme, Dyspnoe und eine hochgradige Beeinträchtigung des Allgemeinzustands auf, ausserdem Herzvergrösserung und ein schwaches systolisches Geräusch. Keine Arrhythmie. Das Ekg ergab Linkshypertrophie, Überleitungs-

zeit normal, S-T in Ableitung 1 und 2 anisoelektrisch, sinkend, T<sub>2</sub> negativ. Blutdruck 170/70.

Während der nächsten Tage wurde die Insulindosis Schritt für Schritt vermindert, und am 9. III. wurde das Insulin ganz ausgesetzt. Im Zusammenhang mit der Insulinverminderung sichtliche Besserung des Allgemeinzustands, das Körpergewicht sank in 9 Tagen von 76,9 auf 69,1 kg, und gleichzeitig verschwanden die Ödeme. Dyspnoe und Herzbeschwerden hörten auf. Nachdem sich gezeigt hatte, dass Pat. Digitalis nicht vertrug, welches Übelkeit und Erbrechen hervorrief, wurde dasselbe durch Coramin ersetzt, wobei die Magenbeschwerden verschwanden und auch die Herzvergrößerung zurückging.

Das Insulin war in diesem Falle ganz entbehrlich, ohne dass die Zuckerausscheidung nennenswert stieg (s. Abb. 53).

Während der ganzen Beobachtungszeit keine Ketonurie. Am 16. III. 36 waren alle Ödeme verschwunden und Pat. subjektiv fast völlig beschwerdefrei. Er konnte nach einigen weiteren Wochen seine Arbeit wiederaufnehmen. Im November d. J. von neuem Verschlimmerung mit Schmerzen in der rechten Schulter, Herzbeschwerden, Abmagerung und Azidose. An diesem Zeitpunkt wurde ein Ca. coli mit Skelettmastasen konstatiert. Pa. verstarb im folgenden Herbst an Karzinom.

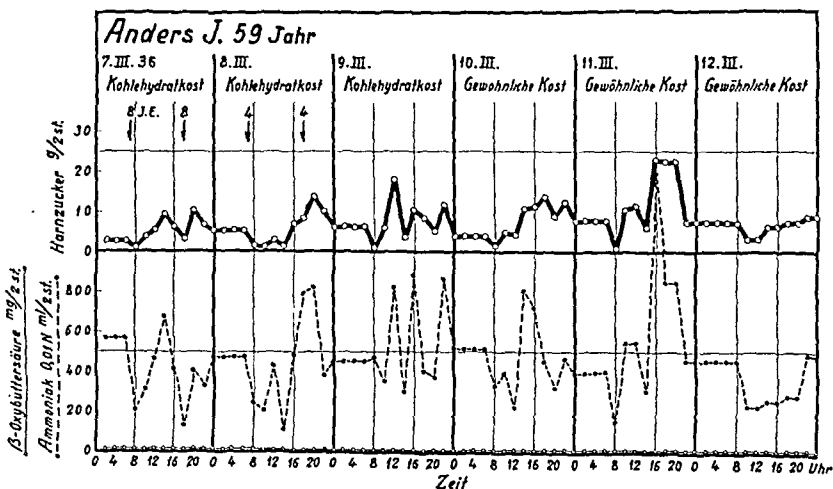


Abb. 53. Ausscheidungsdiagramm bei Diabetes ohne Ketonurie, kombiniert mit Herzfehler. Herzinkompensation im Anschluss an Insulinbehandlung. NB. Keine Zunahme der Zuckerdiurese trotz Aussetzens des Insulins!

*Liegt gleichzeitig mit einem Diabetes ein Mangelzustand vor, dann ist die Beseitigung des letzteren ein unbedingtes Erfor-*

*dernis für eine rationelle Diabetestherapie.* Bei unzulänglicher Insulinwirkung im Organismus muss daher Insulin zugeführt werden. Es ist da nicht möglich, mit irgendeinem diätetischen Regime die *Stoffwechselstörung als solche* günstig zu beeinflussen, wenn sich auch die Zuckerdiurese durch restriktive Massnahmen herabdrücken lässt. Ist die primäre Diabetesursache dagegen nicht eine ungenügende Insulinwirkung, sondern irgendein anderer Mangelzustand oder evtl. eine Regulationsstörung, welche die endogene Insulinproduktion nicht beeinträchtigt, dann wird die Insulinbehandlung irrationell, und sie bleibt da auch mehr oder weniger wirkungslos (vgl. Fall 27 S. 335).

Die Wirkung des Insulins in bezug auf die Zuckerausscheidung schwankt dementsprechend bei den einzelnen Diabetesfällen. Eine bestimmte Beziehung zwischen der Menge des zugeführten Insulins und der Wirkung desselben auf Blutzuckerspiegel und Zuckerdiurese von Fall zu Fall gibt es daher nicht, sofern es sich nicht um einen reinen Insulinmangeldiabetes handelt.

## Regulationsdiabetes

Unter normalen Verhältnissen variiert der Blutzuckerspiegel nur unerheblich und übersteigt auch bei Nahrungsbelastung selten 0,20 %. Wenn man die Blutmasse auf  $7\frac{1}{2}$  l berechnet, beträgt die Gesamtmenge der zirkulierenden Glykose beim Menschen maximal 15 g. Unter der Voraussetzung, dass die biologische Nierenpermeabilität (vgl. S. 183) nicht verändert ist, wird normalerweise die ganze zirkulierende Glykose im Körper zurückgehalten und kann sich am normalen Kohlehydratstoffwechsel beteiligen.

Bei veränderter, erhöhter, biologischer Nierenpermeabilität kann schon eine verhältnismässig geringfügige Blutzuckersteigerung einen Verlust von zirkulierender Glykose verursachen, oder ein solcher kann sogar bei einem normalen Blutzuckergehalt eintreten. Dabei handelt es sich um einen Diabetes vom *renalen Typus*.

Da die biologische Permeabilität der Niere einen integrierenden Faktor bei der Blutzuckerregulation darstellt (vgl. S.

185), ist die renale Diabetesform, mit gesenkter Nierenschwelle, eine Form des Regulationsdiabetes. Abgesehen von dem Zucker- verlust, welcher in schwereren Fällen zu sekundären Kompli- kationen, Kohlehydratverarmung und daniederliegender Gly- kogenbildung, zu führen vermag, kann der Zwischenstoff- wechsel im übrigen relativ ungestört sein.

Da die rhythmische Glykogenbildung der Leber mit einer Tendenz zu periodischen Blutzuckerfluktuationen auch im Hunger einhergeht (vgl. III. Abschnitt), der Blutzuckerspiegel in der Norm aber verhältnismässig konstant ist und sogar bei Nahrungszufuhr nur geringe alimentäre Steigerungen aufweist, die 0,20 % nicht übersteigen, muss der neurohormonale Regu- lationsmechanismus zeitweise einer stärkeren Beanspruchung ausgesetzt sein, wenn die Blutzuckerkurve gleichmässig verlau- fen soll. Es besteht daher normalerweise ein *neurohormonaler Rhythmus*, der mit der periodischen Glykogenbildung inter- feriert und die Wirkungen der wechselnden Kohlehydrataffi- nität der Leber während der verschiedenen Phasen der Tätig- keit dieses Organs auszugleichen bestrebt ist.

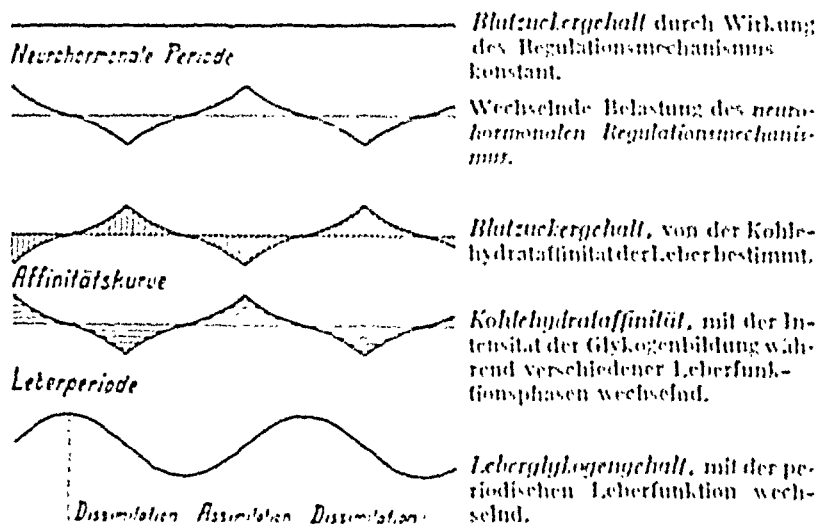


Abb. 51. Schematische Darstellung der periodischen Glykogenbildung der Leber, der Kohlehydrataffinität derselben sowie des interferierenden neurohormonalen Regulationsrhythmus.

Man kann das so ausdrücken, dass die Affinitätskurve der ersten Ableitung der Glykogenbildungskurve entspricht. Dies ist schematisch in der vorstehenden Abbildung dargestellt.

Tritt eine funktionelle Phasenverschiebung zwischen der neurohormonalen Regulationsperiode und der periodischen Glykogenproduktion ein, so bleibt der Blutzuckergehalt nicht länger innerhalb normaler Grenzen konstant. Es kommt da zu einem Diabetes mit abnormen Blutzuckersteigerungen bzw. Blutzuckerfall.

Ein derartiger Diabetes ist der Ausdruck einer *funktionellen Rhythmusstörung*. Sofern es sich nicht um eine dauernde Veränderung im Zwischenstoffwechsel oder um eine irreparable Schädigung des neurohormonalen Regulationsmechanismus handelt, verschwindet dieser Diabetes, wenn die Interferenz der beiden Perioden wieder regelrecht wird.

Ein *transitorischer Diabetes* dieser Art bedeutet daher nicht notwendigerweise eine eigentliche Störung im intermediären Kohlehydratstoffwechsel, nur ein Rhythmusstörung der Blutzuckerregulation.

Für eine normale Blutzuckerregulation ist ein Gleichgewichtszustand zwischen blutzuckersenkenden und blutzuckersteigernden Faktoren erforderlich (Abb. 55).

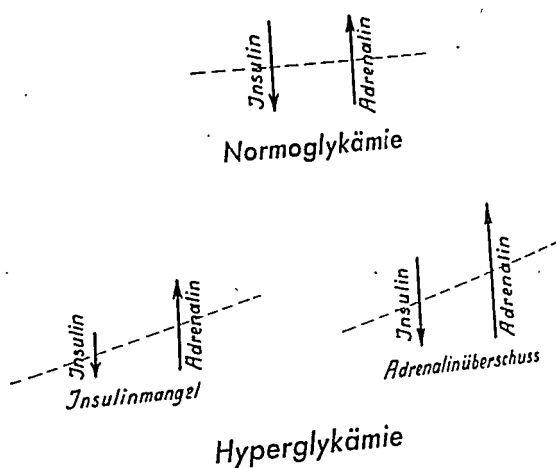


Abb. 55. Schematische Darstellung des Zustandekommens der Hyperglykämie.

Der Blutzuckerspiegel steigt sowohl bei Hypofunktion des blutzuckersenkenden wie bei Hyperfunktion des blutzuckersteigernden Regulationsmechanismus. In beiden Fällen entsteht ein Diabetes. Beruht dieser Diabetes auf einem funktionellen Insulinmangel, so ist die biologische Voraussetzung für die Stoffwechselstörung eine ganz andere, als wenn der diabetische Zustand durch eine funktionelle Mehrleistung des blutzuckersteigernden sympathico-adrenalen Systems bedingt ist.

### Kriterien mangelhafter Insulinwirkung

Da das Insulin einen Hauptfaktor in dem blutzuckersenkenden Regulationssystem bildet, ist die genaue Kenntnis der klinischen Ausfallerscheinungen bei abgeschwächter Insulinwirkung im Organismus eine notwendige Voraussetzung für die richtige Beurteilung eines diabetischen Zustands. Diese Ausfallssymptome lassen sich verfolgen, wenn die Insulindosis bei einem insulinbedürftigen Diabetiker Schritt für Schritt herabgesetzt wird. Dabei zeigt es sich, dass die Wirkung des Insu-

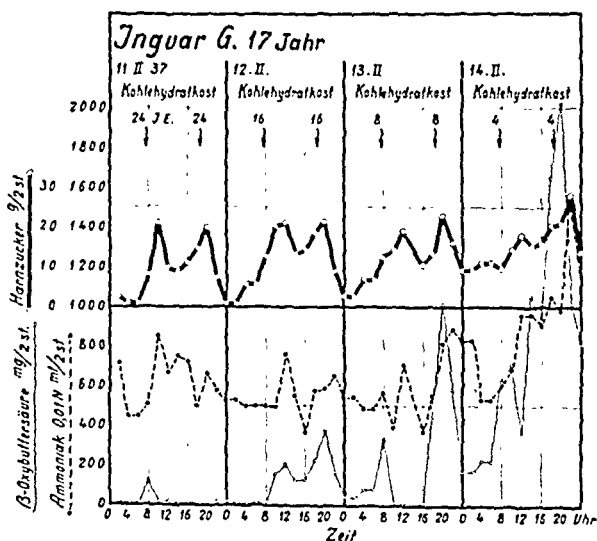


Abb. 56. Bei gleichbleibender Kost aber fallenden Insulinmengen ist die Zuckერიдиурезе relativ konstant; hingegen nimmt die Ketonurie zu.

lins auf die Zuckerdiurese und die Ketonkörperausscheidung nicht dieselbe ist (s. Abb. 56).

Bei ketonkörperbildenden Diabetesfällen folgt die Zuckerausscheidung oft der Kohlehydratzufuhr, auch wenn die Insulindosis vermindert wird. Ist die Kohlehydratmenge in der Kost konstant, dann bleibt auch die Zuckerdiurese bei sukzessiver Insulinverminderung ziemlich unverändert.

Im Gegensatz hierzu steigt bei abnehmender Insulinmenge die Ausscheidung von Ketonkörpern, speziell von  $\beta$ -Oxybuttersäure, mit dem Urin sehr stark und kann im Hunger ohne Insulin maximale Werte erreichen, wobei gleichzeitig die Zuckerausscheidung verschwindet. Die  $\beta$ -Oxybuttersäure lässt dabei oft eine deutlich 24stundenperiodische Ausscheidung erkennen.

Werden Kohlehydrate zugeführt, so nimmt in der Regel die Ketonkörperausscheidung mit steigender Zuckerdiurese und Blutzuckergehalt ab.

Bei mangelnder Kohlehydratzufuhr kann mithin trotz erheblichen Insulindefizits die Zuckerausscheidung verschwinden und der Blutzuckerspiegel normale oder sogar subnormale Werte aufweisen. *Blutzuckergehalt und Zuckerdiurese sind daher kein Indikator für unzulängliche Insulinwirkung.* Demgegenüber ist die  $\beta$ -Oxybuttersäureausscheidung und Ketonämie bei Kohlehydratentziehung ein sichereres Zeichen von Insulinmangel im Organismus.

Im Einklang mit der im VI. Abschnitt gegebenen Darstellung hat man grundsätzlich die beiden regulatorischen Systeme zu berücksichtigen, deren kombinierte Wirkung die normalen Fluktuationen des Blutzuckergehalts bedingt. Das *cerebrall-hypophysäre System* besorgt im wesentlichen die *basale Blutzuckerregulation*, im Hunger allein, bei Nahrungszufuhr gemeinsam mit dem *duodeno-pankreatischen System*, welches letztere bei der *alimentären Blutzuckerregulation* mitwirkt. Diese Regulationssysteme greifen ineinander, und in der Norm muss auch bei Nahrungsbelastung die basale Regulation ungestört verlaufen. Es können jedoch unabhängig voneinander Störungen vorwiegend in dem einen oder anderen System auftreten.

## Störungen in der basalen Blutzuckerregulation

Die Blutzuckerregulation des Grundumsatzes findet durch das zerebral-hypophysäre System statt. Charakteristisch für eine Störung im basalen Regulationssystem ist das Hervortreten abnormer Stoffwechselprodukte auch bei Nahrungsbeschränkung und im Hunger. Mit sinkendem Blutzuckergehalt macht sich dabei in erster Linie die Ketonkörperbildung bemerkbar, ein Zeichen der Veränderung im normalen Fett- und Lipoidstoffwechsel. Steigt der Blutzuckerspiegel bei reichlicherer Nahrungszufuhr, so kann der Lipoidumsatz besser und die Ketonkörperbildung geringer werden, wobei zugleich die Zuckerdiurese zunimmt. Ein Diabetes von diesem Typus wird im folgenden als Diabetes vom Typus A oder A-Diabetes bezeichnet.

Störungen im zerebral-hypophysären Regulationssystem können entstehen durch abnorme, von den Stoffwechsel regelnden Zentren im Gehirn ausgehende Reizimpulse. Auch durch Veränderungen der Hypophysenfunktion oder durch regelwidrige Hormonproduktion in denjenigen endokrinen Drüsen, welche unter dem Einfluss der Hypophyse und des Zwischenhirns stehen, können Störungen zustande kommen. Dabei kann auch infolge von Ausfall von Hormonwirkungen, welche in den Kohlehydratstoffwechsel eingreifen, ein endogener Mangeldiabetes entstehen.

Liegt eine zentrale nervöse Störung von funktioneller Art vor, so können auch andere vegetative Funktionen in Mitleidenschaft gezogen werden. Der diabetische Zustand kann da als ein Teilsymptom einer vegetativen Neurose aufzufassen sein. Bei einem derartigen Regulationsdiabetes können Blutzuckersteigerung und Zuckerdiurese auf einem Reizzustand im sympathico-adrenalen System beruhen.

Diesem Diabetes liegt keine ungenügende Insulinwirkung zugrunde, und er ist infolgedessen oft insulinresistent. Die Insulinbehandlung kann dabei eine Verschlimmerung des Zustands verursachen, da die Insulinzufuhr die Sachlage noch mehr komplizieren und eine regulatorische Gegenreaktion in



einem zuvor überreizten adrenal-vegetativen Regulationssystem auslösen kann.

Eine andere Ursache zentraler Regulationsstörungen ist eine abnorme Wirkung des glandotropen Hormons der Hypophyse. Bei Mangel an insulotropem Vorderlappenhormon tritt eine Atrophie der Langerhansschen Zellinseln mit ungenügender Insulinbildung ein. Dabei entwickelt sich ein hypophysärer Diabetes vom Insulinmangeltypus.

Die hypophysäre Störung kann selbstverständlich mehreren glandotropen Hormone betreffen, wodurch komplizierte Verhältnisse geschaffen werden. Man kann da allerhand Kombinationen begegnen, z. B. mit Hyperthyreosen, Myxödem, Fettsucht, Störung der Sexualfunktionen, Akromegalie, hypophysärem Zwergwuchs usw., welche als Parallelerscheinungen einen bestehenden hypophysären Diabetes komplizieren.

Der Zustand kann dadurch noch verwickelter werden, dass eine von basalen Zentren ausgehende nervöse Störung die Ursache der veränderten Hypophysenfunktion sein kann. Ein Reizzustand oder eine Schädigung in die Funktion der Hypophyse regulierenden Zentren liegt da letzten Endes dem diabetischen Zustand zugrunde. Durch von übergeordneten Gehirnzentren ausgehende reflektorische Impulse können auch die stoffwechselregelnden Zentren beeinflusst werden, wodurch sich die Stoffwechsellage auch unter der Einwirkung psychischer Faktoren ändern kann. Ein hypophysärer Diabetes bietet daher nicht selten ein klinisch sehr wechselndes Bild.

### **Störungen in der alimentären Blutzuckerregulation**

Das duodeno-pankreatische Regulationssystem hat hauptsächlich für die Regulation des Blutzuckergehalts bei Nahrungszufuhr zu sorgen. Liegt eine Störung nur in diesem Regulationssystem vor, ohne primäre Schädigung im Zwischenstoffwechsel, so kann die Kohlehydratverbrennung im Hunger oder bei beschränkter Nahrungszufuhr relativ ungestört sein. Dies zeigt sich u. a. in dem Fehlen der Ketonkörperbildung bei kurzdauernder Nahrungsentziehung. Verschwinden der

Zuckerausscheidung und normalen, möglicherweise leicht erhöhten oder subnormalen Blutzuckerwerten.

Wird in diesem Falle Nahrung in reichlicherer Menge zugeführt, namentlich Kohlehydrate, welche leicht in Glykose übergehen, so tritt dagegen eine abnorme alimentäre Blutzuckersteigerung ein. Der Blutzuckerspiegel steigt dabei auf so hohe Werte, dass die Nieren die Glykose nicht zurückzuhalten vermögen. Es entsteht ein Diabetes mit Glykosurie, aber ohne Ketonkörperausscheidung. Dieser Diabetesstypus wird im folgenden als Diabetes vom Typus B oder B-Diabetes bezeichnet. Infolge des Zuckerverlustes können die Kohlehydrate der Nahrung nicht in entsprechendem Grade ausgenützt werden wie die Fette, welche bei der Verdauung keine Glykose liefern und infolgedessen auch den Blutzuckergehalt nicht in demselben Masse steigern.

In schwereren Fällen wird der Kohlehydratverlust des Organismus so gross, dass die Glykogenbildung darunter leidet. Da muss das Fett im grösserem Umfang in den Dienst des energetischen Stoffwechsels treten und aus diesem Grunde in Kohlehydrat umgewandelt werden. Kommt es hierbei zu Störungen auch in der normalen Umsetzung der Fettsäuren, so werden Ketosäuren gebildet, die in schweren Fällen das Bild eines bedrohlichen Diabetes entstehen lassen können. Da ist es nicht möglich, zu entscheiden, ob es sich anfangs um eine primäre Stoffwechselstörung oder um eine Regulationsschädigung mit hochgradigem Kohlehydratverlust und sekundären Störungen im Fettumsatz gehandelt hatte.

Bei weniger schweren Diabetesfällen ist es möglich, sich durch ein systematisches Studium der Stoffwechselprozesse im Hunger und bei Nahrungszufuhr ein klareres Bild von dem Zustand zu machen. Die Entdeckung der rhythmischen Leberfunktion und des mit dieser verknüpften endogenen Stoffwechselrhythmus gewährt Möglichkeiten, tiefer in den Verlauf der Stoffwechselprozesse einzudringen. Dadurch, dass der Intermediärstoffwechsel nicht kontinuierlich sondern periodisch verläuft, häufen sich bei Störungen in dem normalen Abbauvorgang an verschiedenen Zeitpunkten verschiedene Zwischenprodukte an. Bei ungeschädigter Niere wird der Überschuss

der gebildeten, im Organismus aber nicht weiterumgesetzten Intermediärprodukte zum grossen Teil ausgeschieden. Ausscheidungsdiagramme können daher ein Bild von der Stoffwechselstörung geben, und in ihnen spiegeln sich in hohem Masse die physiologisch-chemischen Vorgänge im Organismus wider. Da das meiste bei ungeschädigten Nieren ausgeschieden wird, vermag das Studium von Blutveränderungen allein nicht denselben klaren Eindruck von der Stoffwechselstörung zu vermitteln wie ein systematisches Studium des Urins. Blut- und Urinuntersuchungen müssen aber einander ergänzen, wenn man ein vollständiges Bild des physiologisch-chemischen Geschehens erhalten soll.

Bei mangelhafter Insulinwirkung macht sich, wie früher hervorgehoben wurde, eine Ketonkörperbildung bemerkbar. Das Studium derselben wird dadurch in hohem Grade erleichtert, dass man die  $\beta$ -Oxybuttersäureausscheidung im Urin quantitativ verfolgt. Ist die Nierenleistung eine gute, so können die Ketosäuren so vollständig ausgeschieden werden, dass sie im Blute grösstenteils fehlen, aber im Urin zu finden sind.

In anderen Fällen kann das Eingreifen der Niere in den Ketonkörperumsatz so gehemmt sein, dass auch bei hochgradiger Ketonämie keine Ausscheidung von Ketonkörpern eintritt (vgl. Tab. 11 S. 235).

### Blutzuckergehalt und Ketonkörperbildung

Verfolgt man den Blutzuckergehalt parallel mit den Ketonkörpern in Blut und Urin, so findet man bei gewissen Diabetesfällen einen erhöhten Ketosäuregehalt in Blut und Urin bei sinkenden Blutzuckerwerten. Dieses Verhalten zeigt hier folgende Tabelle 18 bei drei Diabetikern im Hunger. Nahrungsentziehung bewirkt dadurch bei ketonkörperbildenden Diabetikern oft zugleich mit sinkenden Blutzuckerwerten eine gesteigerte Ketonkörperbildung mit Ketonämie bzw. Ketonurie.

TAB. 18.

$\beta$ -Oxybuttersäure im Blut und Urin bei 3 Fällen von Diabetes im Hunger ohne Insulin bei sinkendem Blutzuckergehalt.

Hunger ohne Insulin bei Säuglingen							
	Zeit	Blut		Zeit	Urin		
		Zucker mg %	$\beta$ -Oxybut- tersäure mg %		Zucker g/2 st	$\beta$ -Oxybutter- säure	
						mg %	mg/2 st
<i>Fall 8.</i> Axel A. ....	9	327	36	8—10	7,0	40	64
	12	271	38	10—12	6,4	30	42
	14	285	36	12—14	4,5	90	126
	16	268	23	14—16	4,7	375	581
	18	247	0 <sup>1)</sup>	16—18	6,0	230	529
				18—20	2,2	260	286
<i>Fall 9.</i> Lisa G. ....	9	97	4	8—10	0	0	—
	12	84	14	10—12	0	0	—
	14	96	12	12—14	0	0	—
	16	66	26	14—16	0	0	—
	18	61	75	16—18	0	25	11
				18—20	0	50	23
<i>Fall 10.</i> Karin H....	9	188	0	8—10	0,7	0	—
	12	174	0	10—12	0	0	—
	14	124	0	12—14	0	0	—
	16	97	13	14—16	0	50	43
	18	94	18	16—18	0	40	100
				18—20	0	60	39

<sup>1)</sup> Null bezeichnet einen nichterhöhten Wert unter 2,0 mg %, wo die angewandte Methode nicht sichere Werte gibt.

Der diabetische Zustand bei den Fällen in Tab. 18 wird aus folgenden Krankengeschichten und Ausscheidungsdiagrammen ersichtlich:

*Fall 8.* Axel A., geb. 1912.

Ein Bruder leidet an Diabetes. — Im November 1933 gelegentlich einer zufälligen Infektion Durstgefühl, starker Hunger und Abmagerung. Es wurde Diabetes festgestellt, Pat. lag 6 Monate im Krankenhaus und erhielt strenge Diät. Insulinbehandlung, 88 I. E. pro Tag, nach und nach Herabsetzung der Dosis.

Es folgt hier das Ausscheidungsdiagramm bei der Beobachtung im März 1942 (Abb. 57).

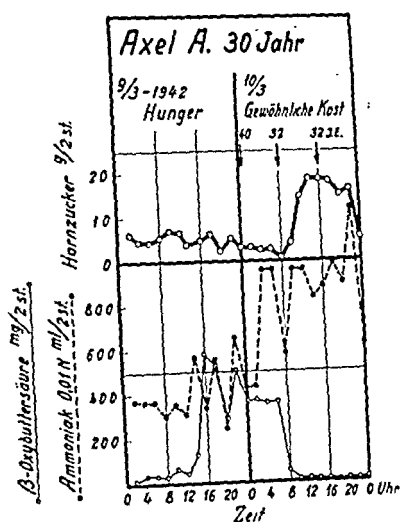


Abb. 57. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes. Zu beachten ist die periodische  $\beta$ -Oxybuttersäureausscheidung während des Hungertages bei sinkender Ketonämie (vgl. Tab. 18), sowie vermehrte  $\beta$ -Oxybuttersäureausscheidung bei sinkendem Blutzuckergehalt. Gute Ammoniakbildung.

Fall 9. Lisa J., geb. 1922.

Keine erbliche Diabetesbelastung. — Im Mai 1940 Müdigkeit, Durst und starke Abmagerung. Diabetes diagnostiziert, 5wöchige Kranken-

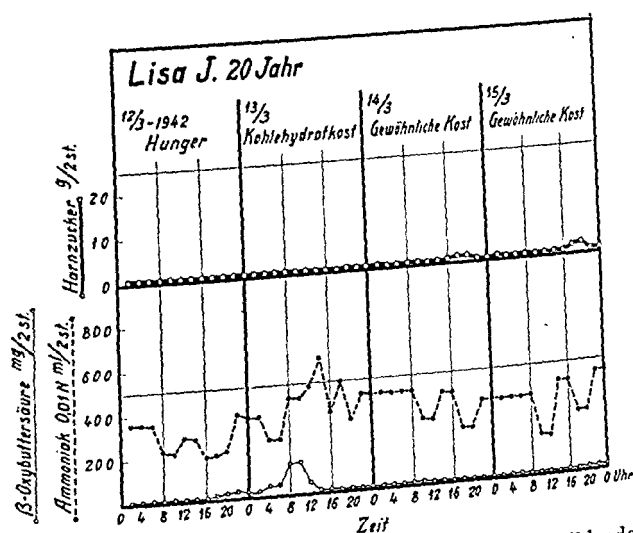


Abb. 58. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes ohne Insulin.

hausbehandlung. Mässig strenge Diät mit 100 g Brot und 75 g Kartoffeln + 20 I. E. Insulin.

Bei der Beobachtung im März 1912 wurde das in Abb. 58 wiedergegebene Ausscheidungsdiagramm erhalten.

Fall 10. Karin H., geb. 1908.

Eine Schwester der Mutter hat Diabetes, sonst keine erbliche Veranlagung. — Bis auf Pleuritis exsudativa 1927 stets gesund gewesen. Im Frühjahr 1911 Gravidität. Abortus imminens mens. III im März. Partus im September; Kind tot. Im August 1911 Durstgefühl und beginnende Abmagerung während der Schwangerschaft. Diabetes im Oktober d. J. konstatiert. Insulinbehandlung im November eingeleitet, 32 I. E. Insulin um 8 Uhr morgens. Oft Insulinbeschwerden.

Der Diabetestypus bei der Beobachtung im Januar 1912 geht aus Abb. 59 hervor.

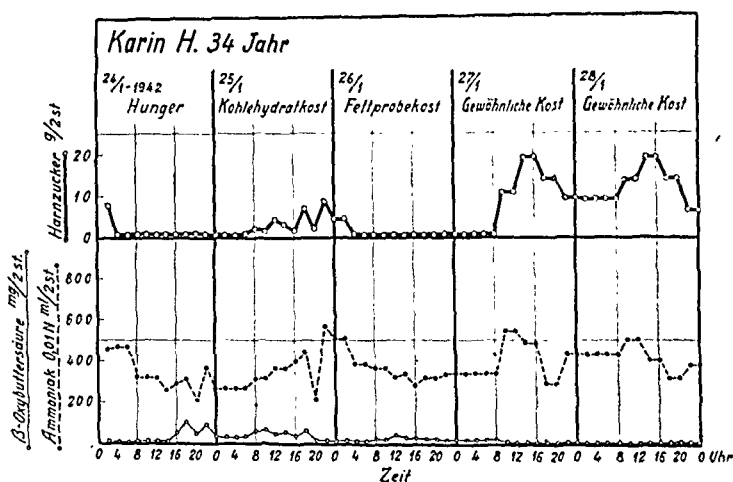


Abb. 59. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes ohne Insulin.

Bei steigendem Blutzuckerspiegel im Zusammenhang mit Kohlehydratzufuhr nimmt dagegen die Ketonkörperbildung ab. Die nächste Tabelle 19 zeigt die Ketonkörperbildung sowie Blut- und Harnzuckergehalt bei zwei Fällen von Diabetes, einerseits im Hunger und andererseits bei einer freien gemischten Normalkost.

TAB. 19.

Gesamtazeton und  $\beta$ -Oxybuttersäure im Blut und Urin sowie Blutzuckergehalt und Zuckerdurese bei 2 Fällen von Diabetes im Hunger ohne Insulin und bei freier Nahrungszufuhr.

	Zeit	Blut			Zeit	Urin				
		Zucker mg %	Gesamt- azeton mg %	$\beta$ -Oxy- butter- säure mg %		Zucker g/2 st	Gesamtazeton		$\beta$ -Oxybutter- säure	
							mg %	mg/2 st	mg %	mg/2 st
<i>Fall 11.</i>										
Ingegerd L.	9	144	8	0 <sup>1)</sup>	8—10	0,4	4	3	20	14
Hunger.	12	122	11	71	10—12	0	13,5	4	55	17
	14	120	0	71	12—14	0	13,5	4	55	16
	16	80	44	5	14—16	0	44	17	65	25
	18	84	9	0	16—18	0	44	16	65	24
<i>freie ge- mischte Kost.</i>										
	9	308	0	4	8—10	5,9	0	—	0	—
	12	346	0	4	10—12	5,4	0	—	0	—
	14	473	0	13	12—14	11,8	0	—	0	—
	16	409	0	0	14—16	22,3	0	—	0	—
	18	506	0	0	16—18	16,2	0	—	0	—
<i>Fall 12.</i>										
Elin K. ....	9	306	2	18	8—10	9,6	70	111	195	312
Hunger.	12	252	14	16	10—12	3,8	27	22	80	64
	14	214	4	30	12—14	1,3	95	76	260	208
	16	195	15	6	14—16	0,7	110	55	295	148
	18	198	2	38	16—18	0	48	63	175	228
<i>freie ge- mischte Kost.</i>										
	9	363	0	21	8—10	7,4	62	74	105	126
	12	499	8	22	10—12	23,7	39	124	65	208
	14	527	4	28	12—14	30,4	29	133	40	184
	16	438	8	30	14—16	32,2	31	142	35	161
	18	652	0	11	16—18	24,1	17	58	30	101

<sup>1)</sup> Null bezeichnet einen nichterhöhten Wert unter 2,0 mg %.

Die Tabelle zeigt die grossen Variationen der Ketonämie von Stunde zu Stunde.

Folgende Krankengeschichten und Ausscheidungsdiagramme beleuchten den Diabeteszustand bei den einzelnen Fällen:

*Fall 11.* Ingegerd L., geb. 1929.

Kein Diabetes in der Familie. Mutter wegen Basedowscher Krankheit operiert. — Im Dezember 1941 zunehmender Durst, starker

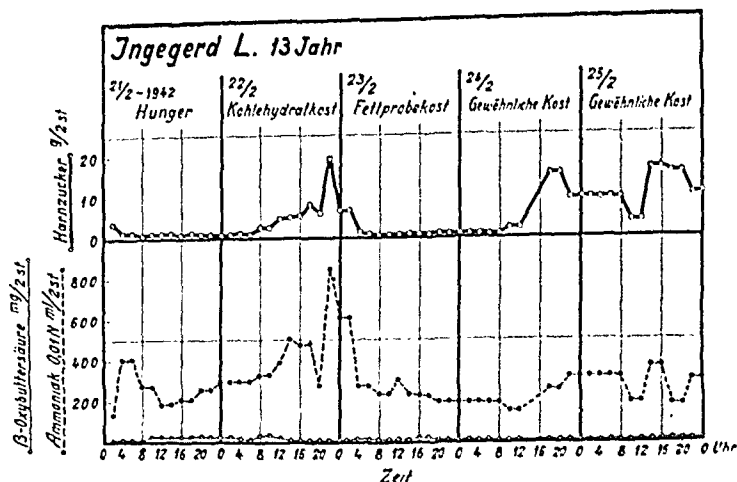


Abb. 60. Ausscheidungsdiagramm bei leichtem ketonkörperbildendem Diabetes ohne Insulin.

Hunger. Geringe Abmagerung und Müdigkeit. Diabetes im Februar 1912 festgestellt. Der Diabetestypus wird von Abb. 60 veranschaulicht.

Fall 12. Elin K., geb. 1890.

Keine erbliche Veranlagung für Diabetes. Von Jugend auf schwächlich gewesen, zeitweise Blutarmut. 1912 Pleuritis exsudativa dextr. Im Frühjahr 1936 Basedowbeschwerden, Herzklopfen, Nervosität,

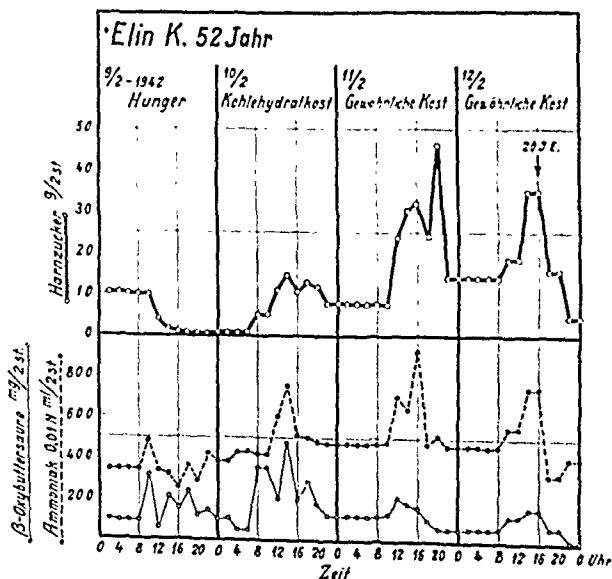


Abb. 61. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes.



Abmagerung und Tremor. Im Frühjahr 1937 Verschlimmerung, Herzvergrößerung; Operation wegen Basedowstruma im März 1937. Im Herbst d. J. Grundumsatz normal, gesund bis Frühjahr 1940. Da Durstgefühl und beginnende Abmagerung, welche im Laufe des Herbstes zunahm. Im November 1940 wurde der Diabetes konstatiert. Vorübergehende Augenbeschwerden. Stationäre Behandlung 3 Wochen mit Diät ohne Insulin. — Bei der Beobachtung im Februar 1942: Länge 165 cm, Gewicht 44,1 kg. Grundumsatz — 16 %. Keine Anämie. Augenhintergrund auf beiden Seiten o. B. Herz: Grenzen 3 + 8 cm. Töne rein. Keine Arrhythmie. Blutdruck 130/80. Pulsfrequenz 80. Der Diabetestypus geht aus Abb. 61 hervor (vgl. Tab. 11 S. 235).

Wenn Störungen im Fettstoffwechsel mit Ketosäurenbildung vorliegen, nimmt diese daher oft mit steigendem Blutzuckerspiegel ab, was darauf hindeutet, dass bei steigendem Blutzuckergehalt die Kohlehydratverbrennung in gewissem Grade gesteigert und möglicherweise die Glykogenbildung erleichtert wird. Die Hyperglykämie ist bei diesen Fällen als eine *Kompensationshyperglykämie* zu betrachten, welche einer bestehenden Störung im Zwischenstoffwechsel entgegenwirkt.

## Der Blutzuckerbegriff

Unter normalen Verhältnissen ist der Zucker im Blute eine Glykose, wahrscheinlich eine  $\alpha$ -Glykopyranose (s. S. 98). Bei dem klinischen Studium der Veränderungen des Blutzuckergehalts beim Diabetes hat man indessen in grossem Umfang die Tatsache übersehen, dass ein mit den üblichen Methoden zur Blutzuckerbestimmung erhaltener Blutzuckerwert keineswegs für Glykose allein gilt, sondern dass es noch Kohlehydratabbauprodukte gibt, welche die Blutzuckerbestimmung in hohem Grade beeinflussen. Unter normalen Bedingungen, wo diese Abbauprodukte zum grössten Teil weiterumgesetzt werden und sich nicht im Blute anhäufen, gibt die Blutzuckerbestimmung den wirklichen Glykosegehalt des Blutes ziemlich sicher wieder. Beim Diabetes mit gestörtem Kohlehydratabbau dagegen kann der Blutzuckerwert in gewissen Fällen recht

irreführend werden, und er ist da kein Massstab für den wirklichen Glykosegehalt des Blutes. Der Blutzuckerwert ist daher beim Diabetes mit allergrösster Vorsicht zu beurteilen.

Es gibt verschiedene Methoden zur Bestimmung des Glykosegehalts des Blutes. Die klinisch gewöhnlich angewandten Mikromethoden gründen sich auf das Reduktionsvermögen der Glykose. Dieses ist an die *Aldehydgruppe* des Hexosemoleküls gebunden. Sind andere Aldehyde anwesend, so reduzieren auch diese. Infolgedessen sind die chemischen Bestimmungsmethoden, welchen Reduktionsverfahren zugrunde liegen, nicht für Glykose spezifisch.

Bei den eher brauchbaren Reduktionsmethoden, z. B. der von *Hagedorn-Jensen*, werden flüchtige Aldehyde, beispielsweise Azetaldehyd, vor der Reduktion abgedampft und alle stickstoffhaltigen reduzierenden Substanzen mit dem Eiweiss ausgefällt. Unter normalen Verhältnissen wird daher der Reduktionswert zu einem wahrscheinlichen Mass für den Glykosegehalt des Blutes, also für den Blutzuckergehalt. Sonstige reduzierende, niedermolekulare Kohlehydrate, Diosen, Triosen usw., werden normalerweise in so grossem Umfang umgesetzt, dass man mit ihrem Vorkommen im Blut im Vergleich zur Glykose nicht zu rechnen braucht.

Bei Störungen im Kohlehydratstoffwechsel hingegen können einfachere Kohlehydrate wie Diosen, Triosen usw. und andere reduzierende Substanzen, z. B. Glykolaldehyd, Glycerinaldehyd, Glyoxyl u. a. m. angehäuft werden, welche beim Erwärmen des Blutes nicht flüchtig sind und auch nicht mit dem Bluteiweiss ausgefällt werden, wodurch sie bei der Blutzuckerbestimmung in hohem Grade störend wirken. Nur insofern als die reduzierenden Aldehydgruppen an Hexosemoleküle gebunden sind gibt der Reduktionswert einen Massstab für den Glykosegehalt des Blutes ab.

Bedient man sich anderer, für Hexosen spezifischer Glykosebestimmungsmethoden, so erhält man bei Störungen im Kohlehydratabbau nicht selten ganz andere Resultate als mit Reduktionsmethoden. Eine spezifische Hexosenreaktion ist die Bildung von Oxymethylfurfurol, welches nach Koppelung an beispielsweise Orzin eine stark gefärbte Verbindung liefert, die

zur quantitativen Hexosenbestimmung anwendbar ist (vgl. S. 97).

Bei vergleichenden Blutzuckerbestimmungen mit der Orzinmethode und z. B. der Hagedorn-Jensenschen Methode — jene Zucker mit 6 kettenförmig gelagerten Kohlenstoffatomen im Molekül nachweisend, diese freie reduzierende Aldehydgruppen — erhält man beim Diabetes wechselnde Resultate, was die hier folgende Tab. 20 mit Bestimmungen an vier verschiedenen Diabetesfällen veranschaulicht.

TAB. 20.

Blutzuckergehalt bei Diabetes vom Typus A und B im Hunger ohne Insulin und bei freier Nahrungszufuhr, nach Hagedorn und mit der Orzinmethode bestimmt.

Diabetestypus	Hunger						freie Kost							
	Blutzucker					Urin		Blutzucker					Urin	
						Zucker	$\beta$ -Oxybutter-säure						Zucker	$\beta$ -Oxybutter-säure
						8—20	8—20						8—20	8—20
	Zeit	9	12	14	16	18		9	12	14	16	18		
		mg %	mg %	mg %	mg %	mg %	g	mg %	mg %	mg %	mg %	mg %	g	mg
<b>A-Diabetes:</b>														
1. Ester H.														
Hagedorn.		125	110	99	64	41	3,5	228	176	153	178	160	172	19,9
Orzin ....		0	0	0	0	0			224	—	267	229	300	0
2. Helvi K.														
Hagedorn.		195	205	189	158	174	45,1	6078	197	258	301	285	291	170,6
Orzin ....		425	329	396	369	334			0	338	340	329	0	0
<b>B-Diabetes:</b>														
3. Hans B.														
Hagedorn.		135	131	119	117	131	5,5	0	141	228	240	277	231	85,9
Orzin ....		279	131	135	79	92			221	300	241	350	259	0
4. Josef H.														
Hagedorn.		155	120	141	101	78	3,3	0	242	245	277	183	245	91,9
Orzin ....		115	71	163	102	75			338	391	442	257	348	0

Es folgen nun die Krankengeschichten und Diagramme zur obigen Tabelle:

Fall 13. Ester H., geb. 1892.

Kein Diabetes in der Familie. — 1917 wegen Extrauterin gravidität, 1926 wegen Appendizitis operiert, dabei rechtsseitige Ovariectomie. 1931 Operation wegen Tumor recti, bei der auch der linke Eierstock entfernt wurde. 1934 Nephrolithiasis und Pyelitis. Seit 1931 zeitweise Durst und lästiges Hautjucken. In dieser Zeit wurde zufällig Zucker im Urin festgestellt. — Im Dezember 1939 Influenza mit hohem Fieber. Während dieser Zeit starker Durst. Im Frühjahr 1940 zunehmende Müdigkeit, Abmagerung und zeitweise Schwindel. Der Diabetes wurde erst im Juni 1940 diagnostiziert. Dreiwöchige Krankenhausbehandlung, wobei Insulintherapie mit wechselnden Mengen eingeleitet wurde, bis 48 I.E. Zinkinsulin. Ziemlich freie Kost. Periodenweise leichte Besserung, aber im Mai 1941 wieder zunehmende Müdigkeit und Schwindel. Wieder 2 Wochen stationäre Behandlung, dabei Änderung der Insulindosierung auf 12 I.E. Zinkinsulin + 12 I.E. gewöhnliches Insulin morgens, sowie strengere Diät. Die Insulindosis wurde allmählich auf 28 I.E. Zinkinsulin + 10 I.E. gewöhnliches Insulin einmal täglich erhöht. Nach wie vor starkes Schwächegefühl, Parästhesien in den Armen, zeitweise Doppelsehen. Die Patientin musste einige Male wegen schweren Schwindels zu Bett liegen.

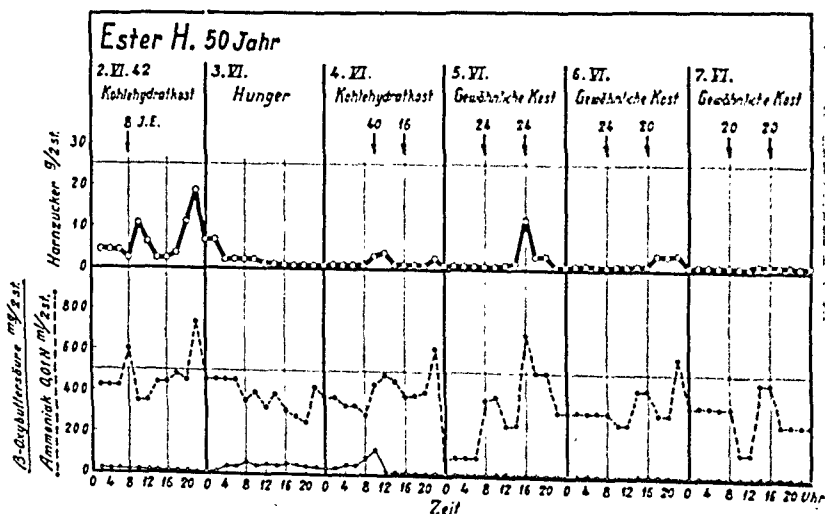


Abb. 62. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes. Blutzuckerbestimmungen am 3. VI. im Hunger und am 9. VI. bei gewöhnlicher Kost und Insulin 16 + 16 I.E. (vgl. Tab. 20).

Bei eingehender Untersuchung im Juni 1942 wurde neben einem Mitralklappenfehler ohne Inkompressionserscheinungen und mit normalem Ekg eine leichte Anämie (Hb. 92 % (Sicca), rote Blutkörperchen 3,98 Mill., weisse 5500) festgestellt, sowie ausgedehnte

Fettgewebsatrophien lateral an beiden Oberschenkeln im Gebiet der Insulininjektionen. Der Diabetestypus wird aus dem Ausscheidungsdiagramm (Abb. 62) ersichtlich.

Wie aus Tab. 20 hervorgeht, wurde bei der spezifischen Hexosebestimmung im Hunger keine Glykose im Blute gefunden, obwohl das Hagedornsche Verfahren verhältnismässig normale Reduktionswerte ergeben hatte.

Fall 11. Helvi K., geb. 1924.

Keine erbliche Veranlagung für Diabetes. — Im Januar 1929 zunehmender Durst und Müdigkeit, aber keine Abmagerung. Diabetes im März d. J. konstatiert. Stationäre Behandlung 3 Monate mit strenger Fett-Gemüsediat + 65 g Brot. Der Durst verschwand, und auch der Appetit wurde geringer. Zu Hause 2 Monate Ruhe, aber nach wie vor starke Müdigkeit. Vom August d. J. an wieder  $4\frac{1}{2}$  Monate Krankenhausbehandlung. Während dieser Zeit wurde die Insulintherapie eingeleitet. Bei der Entlassung  $32 + 32$  I.E. Insulin, weiter strenge Diät. Im Laufe der nächsten Jahre noch achtmal stationäre Behandlung, jedes Mal 5–6 Wochen, zuletzt Januar–Februar 1940. Seit 1938 etwas freiere Diät.

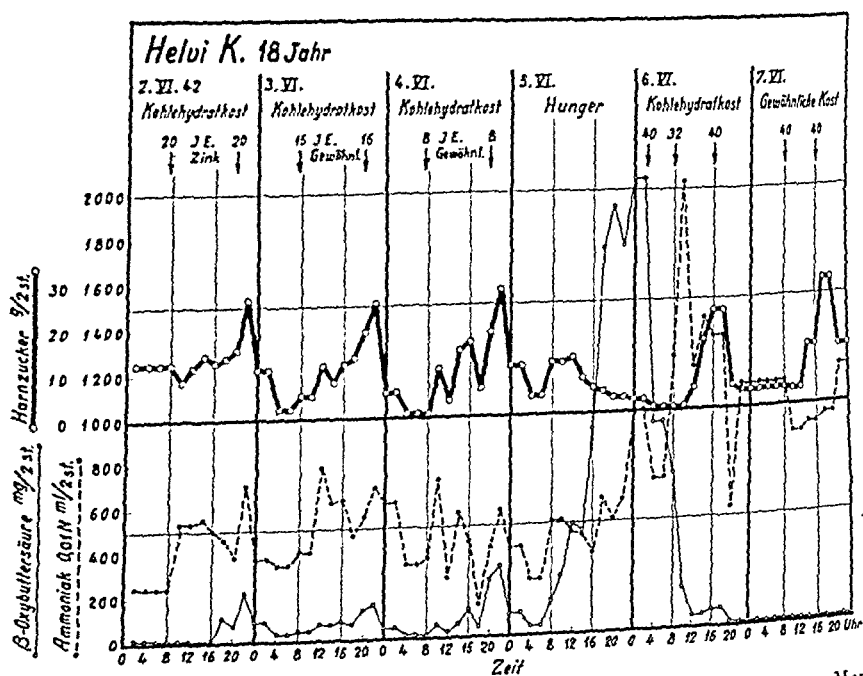


Abb. 63. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes. Man beachte, dass in diesem Fall Zinkinsulin nicht bessere Wirkung gibt als gewöhnliches Insulin. Blutzuckerbestimmungen am 5. VI. im Hunger ohne Insulin und am 11. VI. bei gewöhnlicher Kost und Insulin  $36 + 40$  I.E. (vgl. Tab. 20).

Bei der Beobachtung im Juni 1912 zeigte das Ausscheidungsdiagramm den aus Abb. 63 hervorgehenden Diabetestypus.

Man sieht in Tab. 20, dass die Reduktionswerte des Blutes etwas erhöht waren, aber ausserdem lag ein erheblicher Überschuss an nicht reduzierenden Hexosen vor.

Fall 15. Josef H., geb. 1887.

Die Mutter des Patienten leidet an Altersdiabetes mit Gangrän, ein älterer Bruder ist an Diabetes gestorben. — Im Herbst 1937 wurde zufällig Zucker im Urin festgestellt. Seitdem ab und zu Zucker in kleineren Mengen, niemals Azeton. Sonst munter, kein Durstgefühl. Hat in den letzten fünf Jahren von 79 bis 71 kg abgenommen. Im letzten Jahre zeitweise müde und nervös infolge von Arbeitsüberlastung, auch leichte Herzbeschwerden, Kurzatmigkeit bei Anstrengungen und Herzunruhe. Bei der Beobachtung im Juni 1912: Blutdruck 140/80. Pulzfrequenz 76. Herz: Grenzen 4 + 8 cm. Töne dumpf, systolisches Geräusch über dem ganzen Herzen. Ekg weist Anzeichen einer leichten Kardiosklerose auf.

Das Ausscheidungsdiagramm findet man in Abb. 61.

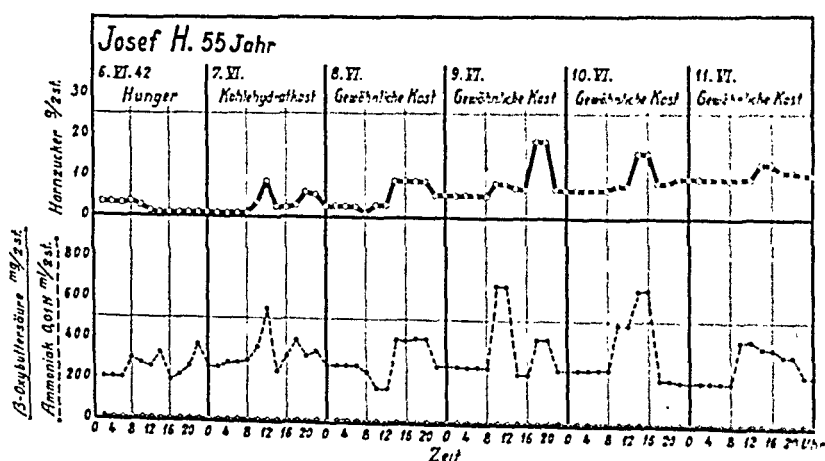


Abb. 61. Ausscheidungsdiagramm bei nicht ketonkörperbildendem Diabetes. Blutzuckerbestimmungen am 6.VI. im Hunger und am 13.VI. bei gewöhnlicher Kost ohne Insulin (vgl. Tab. 20).

Fall 16. Hans B., geb. 1883.

Keine erbliche Diabetesbelastung. — Seit 1918 zeitweise hochgradig neurasthenisch, Müdigkeit, Kopfschmerzen, spastische Magenbeschwerden, Schweissausbrüche, mitunter Schlaflosigkeit. Seit Herbst 1910 periodenweise heftiges Hungergefühl, ohne nennenswerten Durst. Beginnende Abmagerung. Diabetes im Frühjahr 1911 konstatiert.

Das Ausscheidungsdiagramm vom Juni 1942 zeigt den Diabetestypus (Abb. 65.)

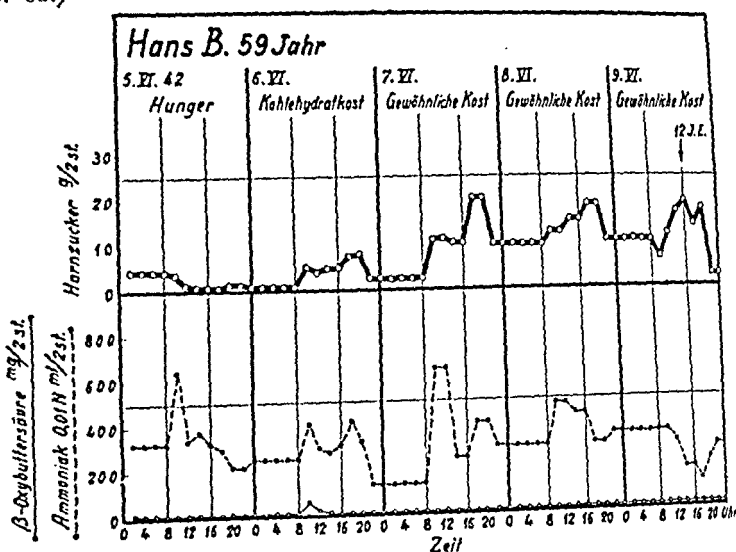


Abb. 65. Ausscheidungsdiagramm bei schwach ketonkörperbildendem Diabetes. Blutzuckerbestimmungen am 5. VI. im Hunger und am 9. VI. bei gewöhnlicher Kost und Insulin 12 I.E. um 16 Uhr (vgl. Tab. 20).

Die spezifische Hexosenbestimmungsmethode mit Orzin liefert beim Diabetes manchmal Werte, welche mit den bei Anwendung der Reduktionsmethode nach Hagedorn-Jensen erhaltenen übereinstimmen, aber oft bestehen beträchtliche Abweichungen. Auch bei normalen Reduktionswerten kann die Orzinmethode das Fehlen freier Hexosen im Blute anzeigen. Dies beruht darauf, dass sich beim gestörten Kohlehydratstoffwechsel reduzierende Abbauprodukte, Glycerinaldehyd und andere Substanzen, im Blute anhäufen und die Glykose als energiebildendes Verbrennungsmaterial bei den oxydo-reduktiven Prozessen im Organismus ganz oder teilweise ersetzen.

Beim Vorkommen von polymerisierten Hexosemolekülen, z. B. von Glykogen, welche im Zusammenhang mit der Orzinreaktion hydrolysiert werden, gibt diese Methode auch einen Ausschlag für dieselben und liefert dabei höhere Werte als die Reduktionsmethoden. Dies ist auch der Fall, wenn die Aldehydgruppe der Hexosemoleküle blockiert ist, z. B. mit Phosphorsäure bei der Hexosediphosphorsäure oder dem Hexosephosphat (Cori-Ester, vgl. S. 102).

Wie aus Tab. 19 S. 288 ersichtlich wird, findet man auch beim Diabetes bisweilen höhere Orzinwerte als mittels der Hagedorn-Jensenschen Methode bestimmte Reduktionswerte.

Der Begriff »Blutzucker« muss daher beim Diabetes mit grösster Zurückhaltung aufgefasst werden. Man muss sich zunächst Klarheit darüber verschaffen, ob Hexosen, Kohlehydrate mit 6-Kohlenstoffketten, oder Aldehydgruppen enthaltende Kohlehydrate mit wechselnder Kohlenstoffkette bestimmt werden sollen. Zum Nachweis der ersteren dient die Eigenschaft der Hexosen, Oxymethylfurfurol zu bilden, zu dem der letzteren geeignete Reduktionsmethoden. Mit verschiedenen Methoden erzielte Blutzuckerwerte lassen sich nicht miteinander vergleichen. Diese Tatsache ist von grundlegender Bedeutung, wenn es sich um die Klärung der Kohlehydratstoffwechselstörung beim Diabetes handelt.

Neben Blutzucker kommt auch Glykogen normalerweise im Blut vor. Dasselbe ist einmal an Eiweiss gebunden als Desmoglykogen, welches der Eiweissniederschlag bei der Blutzuckerbestimmung mit sich reisst, sodann in löslicher Form als Lyoglykogen vorhanden; letzteres findet man nach vorangehender Hydrolyse als Blutzucker wieder. Ohne Hydrolyse macht sich das Glykogen bei gewöhnlichen Blutzuckerbestim-

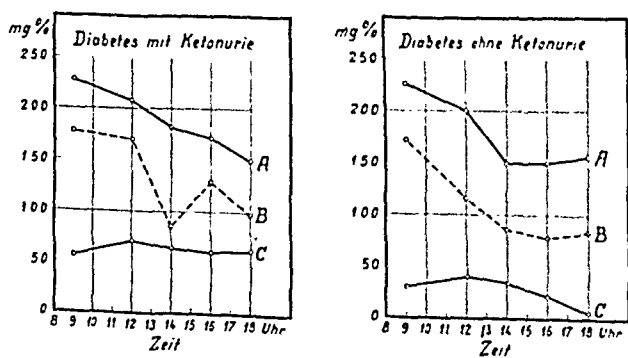


Abb. 66. Blutzucker- und Glykogengehalt des Blutes bei Diabetes mit und ohne Ketonurie im Hunger ohne Insulin.

- A Blutzucker, Bestimmung nach Hagedorn-Jensen.  
 B Blutzucker, als Oxymethylfurfurol mit Orzin bestimmt.  
 C Glykogen (Hexose nach Hydrolyse des Glykogens als Oxymethylfurfurol bestimmt).



mungen nicht bemerkbar. Auch das Blut des Diabetikers enthält Glykogen. Abb. 66 zeigt Mittelwertkurven bei 7 Fällen von ketonkörperbildendem Diabetes; der Blutzuckergehalt ist einerseits mit dem Hagedornschen Verfahren und andererseits mit einer spezifischen Hexosenbestimmungsmethode mittels Orzin festgestellt. Gleichzeitig wurde der Glykogengehalt des Blutes ermittelt.

Auffallend ist der trotz sinkender Blutzuckerwerte, namentlich Reduktionswerte, konstante Glykogengehalt. Beachtung verdient ferner das Hexosemaximum, welches bei der Orzinbestimmung um 16 Uhr ohne nennenswerte Erhöhung des Reduktionswerts hervortritt. In Abb. 66 findet man auch entsprechende Mittelwertkurven bei 3 Fällen von Diabetes ohne Ketonkörperbildung. Hier liegt der Glykogengehalt tiefer und sinkt während des Hungertages, nachdem der — sowohl nach Hagedorn wie mit der Orzinmethode bestimmte — Blutzucker einen asymptotischen Minimalwert erreicht hat.

Wo im folgenden von »Blutzucker« die Rede ist, hat man hierunter die im klinischen Schrifttum bislang gebräuchlichen Reduktionswerte zu verstehen, welche mit der Hagedorn-Jenssenschen Methode erhalten werden. Ob es sich hierbei um Glykose oder andere reduzierende Kohlehydratabbauprodukte handelt, welche die Reduktion bewirken, lässt sich ohne eingehendere Analyse der Kohlehydrate des Blutes im Einzelfalle nicht entscheiden.

## Endogener Rhythmus und intermediäre Stoffwechselprozesse

Der ständige Wechsel der physiologisch-chemischen Vorgänge, mit zeitweiliger Anhäufung intermediärer Produkte, gewährt beim Diabetes eine Möglichkeit, den Verlauf des Stoffwechsels genauer zu verfolgen. Bei ungeschädigter Nierenfunktion wird nämlich jeglicher Überschuss an nicht umgesetzten Stoffwechselerzeugnissen entweder in unveränderter Form oder in durch Kopplung an andere Substanzen stabilisiertem und entgiftetem Zustande im Urin ausgeschieden. Eine

systematische Urinuntersuchung kann daher ein Bild von den Prozessen im Organismus geben und dadurch Aufschlüsse über die Stoffwechselstörung und deren eventuellen Angriffspunkt in der Kette der biochemischen Reaktionen liefern.

Im folgenden werden einige orientierende Beispiele dafür angeführt werden, wie der endogene Rhythmus beim Studium von Stoffwechselvorgängen bei Diabetes mellitus zum Vorschein kommt.

### Die Ausscheidung der Phosphorsäure

Bei Störungen im Phosphorylierungsprozess ist der Körper nicht imstande, sich die zur Verfügung stehende Phosphorsäure zunutze zu machen. Diese wird da in grösserer oder geringerer Menge ausgeschieden. Die Phosphorsäure zeigt wie die  $\beta$ -Oxybuttersäure eine mehr oder weniger stark hervortretende 24stundenperiodische Ausscheidung. Die Phosphorsäureausscheidung wird während der Wirkung des Insulins geringer, gleichzeitig mit dem Verschwinden der Ketonurie (Abb. 67, vgl. Abb. 68). Bemerkenswert ist, dass die Zuckerausscheidung dabei nicht stets in entsprechendem Grade abnimmt. Auch der Stickstoff, welcher ebenfalls in vielen Fällen eine deutlich 24stundenperiodische Ausscheidung erkennen lässt, folgt nicht der Phosphorsäureidiurese, und die im Urin vorhandene Menge desselben geht nicht infolge der direkten Wirkung des Insulins in gleichem Masse zurück wie die der Phosphorsäure, was ebenfalls aus Abb. 67 ersichtlich wird.

Lässt die Insulinwirkung bei einem Diabetiker mit Ketonkörperbildung im Hunger nach, so steigt die Ausscheidung der Phosphorsäure parallel der erhöhten  $\beta$ -Oxybuttersäureausscheidung. Die Zuckerausscheidung nimmt dagegen nicht zu. Dies deutet darauf hin, dass das Insulin beim Verschwinden des Zuckers nicht unmittelbar beteiligt ist, sondern womöglich sekundär in den eigentlichen Zuckerumsatz eingreift. Der primäre Angriffspunkt des Insulins ist daher wahrscheinlich anderswo zu suchen, möglicherweise in engerer Verknüpfung mit dem Fettstoffwechsel (vgl. Abb. 10 S. 38).

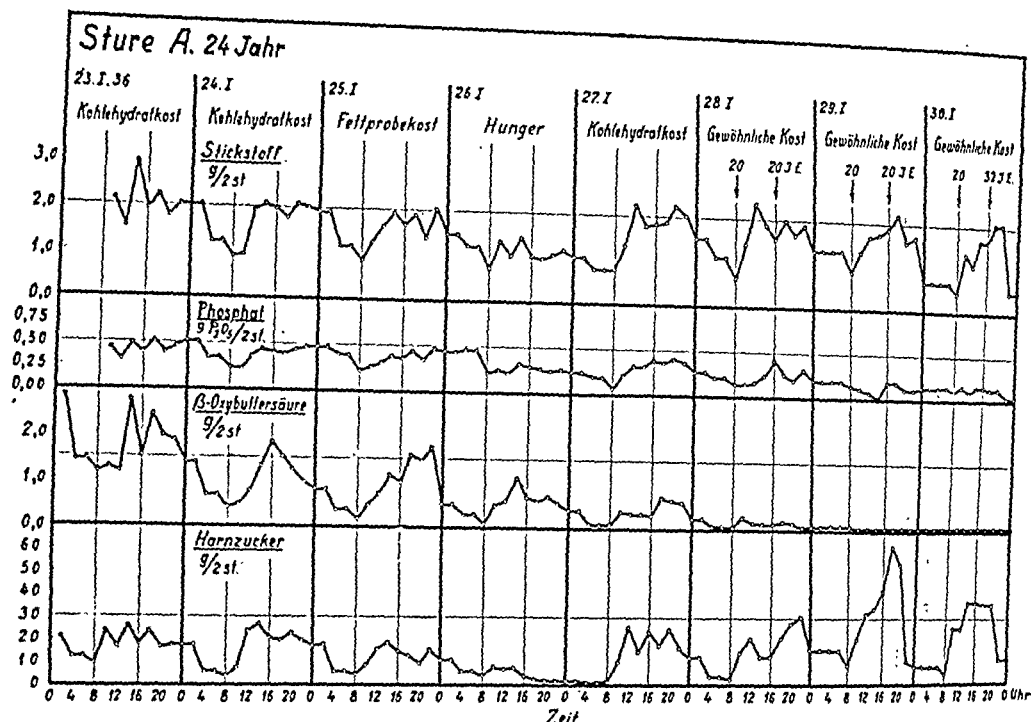


Abb. 67. Ausscheidung von Gesamtstickstoff, Gesamtphosphor,  $\beta$ -Oxybuttersäure und Harnzucker bei ketonkörperbildendem Diabetes während Kohlehydrat-Fettkost, Hunger und gemischter freier Kost mit Insulin. Man beachte die etwas wechselnde Ausscheidungsperiodizität. Stickstoffausscheidung relativ unbeeinflusst, im Hunger etwas herabgesetzt. Kein nennenswerter Einfluss des Insulins. Phosphorausscheidung bei Ketonurie gesteigert und mit dem Verschwinden der  $\beta$ -Oxybuttersäure durch die Insulinwirkung abnehmend. Bei Einleitung der Insulinbehandlung und Erweiterung der Diät steigende Zuckerdiurese trotz Nachlassens der Phosphorausscheidung und Verschwindens der Ketonurie.

Gleichzeitig mit der zunehmenden Ketonurie wird auch Cholin in steigender Menge ausgeschieden (Abb. 68). Hierin liegt die Andeutung eines Zusammenhangs zwischen Ketosäurenbildung und Phosphatidumsetzung (vgl. S. 131).

Die bei Ketonurie vermehrte Phosphatausscheidung bringt die Pufferkapazität des Urins zum Steigen. Dadurch wird die Ausscheidung saurer Produkte erleichtert (s. S. 232). Bei Ketonkörperbildung wird daher die erhöhte Phosphatausscheidung zu einem für die Säuren-Basenregulation des Organismus günstigen Faktor, mit dessen Hilfe sich der Körper grösserer Ketosäuremengen ohne Nierenschädigung durch die Harnwege zu entledigen vermag.

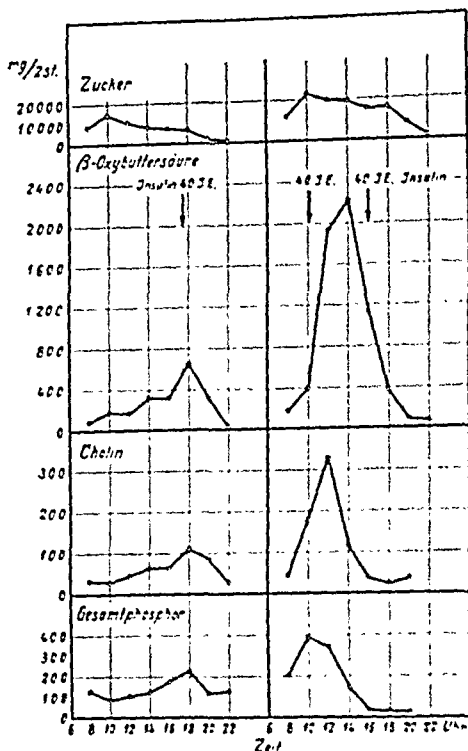


Abb. 68. Ausscheidung von Gesamtphosphor, Cholin,  $\beta$ -Oxybuttersäure und Harnzucker bei 2 Fällen von ketosäurebildendem Diabetes im Hunger sowie Wirkung von Insulin. Bei hochgradiger Ketonkörperbildung (rechtes Diagramm) wird zuerst die Phosphor-, dann die Cholin- und zuletzt die  $\beta$ -Oxybuttersäureausscheidung von 40 I. E. Insulin beeinflusst. Der Einfluss auf die Zuckerausscheidung ist nicht dementsprechend.

Eine 24stundenrhythmische Phosphorsäureausscheidung wird auch beim Diabetes ohne Ketonurie beobachtet, und zwar im Hunger ebenso wie bei Nahrungszufuhr. Abb. 69 zeigt die Zucker-, Gesamtstickstoff- und Gesamtphosphorsäureausscheidung bei einem Fall von nicht ketonkörperbildendem Diabetes. Die periodische Phosphorausscheidung weicht in diesem Falle teilweise von der Zucker- und Stickstoffausscheidung ab.

Fall 17. Alvar S., geb. 1891.

Vater und eine Schwester desselben leiden an Diabetes. Pat. war früher stets gesund und ziemlich mager bis 1918. Begann da zuzunehmen (20 kg in 2 Jahren bis 92 kg). Im Herbst 1920 Müdigkeit und beginnendes Durstgefühl. Damals etwas Zucker

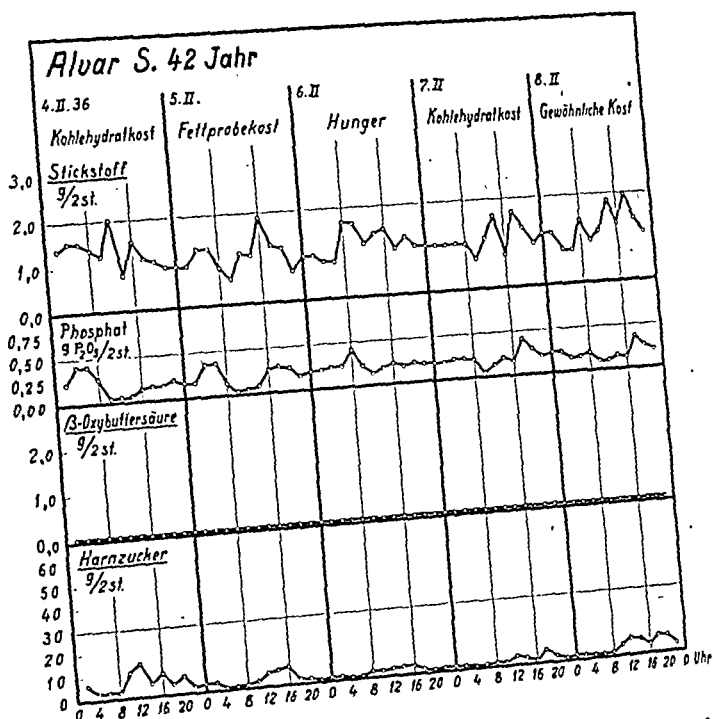


Abb. 69. Ausscheidung von Stickstoff, Phosphor und Urinzucker bei nicht ketonkörperbildendem Diabetes. NB. Phosphor- und Stickstoffausscheidung auch im Hunger nicht verändert und zeigen wie der Harnzucker ebenfalls eine periodische Ausscheidung.

im Urin. Keine Azidose. Allmähliche Besserung, langsame Gewichtsabnahme (10 kg in den folgenden 5 Jahren). Im Herbst 1927 wieder Müdigkeit und leichter Durst. Das Gewicht war im letzten Halbjahr um 5 kg gestiegen. Bei mässig strenger Diät besserte sich der Zustand, und Pat. fühlte sich bis Dezember 1935 wohl. Dann im Zusammenhang mit Überanstrengung bei der Arbeit zunehmendes Schwächegefühl und Durst, besonders am Vormittag, sowie lästiger Harndrang. Schlaf unruhig, Nachtschweisse. Nervös, unregelmässige Darmfunktion. 3.II.1936 Länge 175 cm, Gewicht 88,5 kg. Blutdruck 170/90. Herz und übrige Organe o. B.

Die 24stundenperiodische Phosphorausscheidung kann daher nicht lediglich an eine periodische Ketonkörperbildung gebunden sein, sondern muss an noch andere intermediäre Stoffwechselvorgänge beim Diabetes anknüpfen. Ein Teil der Phosphorumsetzung spielt sich wahrscheinlich in den roten Blutkörperchen ab.

## Blutzuckergehalt und Blutphosphor

Erst nach Phosphorylierung des Hexosemoleküls ist ein weiterer Abbau möglich. Blutzuckergehalt und Phosphorgehalt stehen daher in einem gewissen Abhängigkeitsverhältnis voneinander. Die roten Blutkörperchen besitzen dabei Bedeutung für den Phosphorsäureumsatz. In diesen findet sich u. a. die von Warburg nachgewiesene Codehydrase II, durch deren Wirkung das Glycerinaldehydphosphat ein weiteres Molekül Phosphorsäure unter Bildung von 1-3-Diphosphoglyzerinsäure aufnehmen kann (vgl. S. 109).

Die weitere Umwandlung der Diphosphoglyzerinsäure ist eine notwendige Vorbedingung für einen normalen Verlauf des Kohlehydratabbaus (vgl. IV. Abschnitt). Wird dieser Prozess gestört, dann kann sich Phosphorsäure in den Blutkörperchen ansammeln. Bei Diabetes mit schweren Störungen im Kohlehydratstoffwechsel kann es daher in gewissen Fällen zu einer Anhäufung von Phosphorsäure in den roten Blutkörperchen kommen. Dies ist bei Diabetesformen mit Störungen des Phosphorylierungsprozesses der Fall. Abb. 70 zeigt Mittelwertkurven von 8 Diabetesfällen, bei welchen der Gesamtphosphorge-

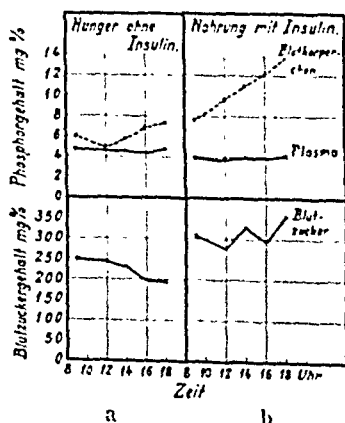


Abb. 70. Verteilung des Phosphors auf Blutkörperchen und Plasma bei Diabetes (Mittelwerte von 8 Fällen).  
 a Im Hunger ohne Insulin.  
 b Bei Nahrungszufuhr mit Insulin.

halt der Blutkörperchen und des Plasmas teils im Hunger und teils bei Nahrungszufuhr bestimmt wurde. Im letzteren Falle erfolgt eine merkbare Zunahme des Gesamtphosphors in den Blutkörperchen und gleichzeitig sinkt derselbe im Blutplasma, was darauf hindeutet, dass die Blutkörperchen beim Phosphorumsatz im Anschluss an Nahrungszufuhr speziell beteiligt sind.

Bei Ketonurie tritt eine vermehrte Phosphorausscheidung auf (Abb. 68). Auch eine zeitweise gesteigerte Phosphoranhäufung in den Blutkörperchen macht sich beim ketonkörperbildenden Diabetes bemerkbar. In Abb. 71 ist der Gesamtphosphorgehalt der Blutkörperchen und des Plasmas im Hunger wiedergegeben, einerseits bei Diabetes ohne Ketonurie (Mittelwertkurve von vier Fällen), andererseits bei einem Fall von Diabetes mit starker Ketonurie ohne Insulin. Gleichzeitig mit einer Senkung des Phosphorgehalts im Blutplasma tritt im letzteren Falle eine maximale Phosphoranhäufung in den Blutkörperchen um 14 Uhr ein, wonach der Phosphorgehalt wieder sinkt. Bei nicht ketonkörperbildendem Diabete fehlt dieses Phosphormaximum. Dies ist ein Hinweis auf eine Störung des Phosphorumsatzes der Blutkörperchen bei Ketonurie. Das

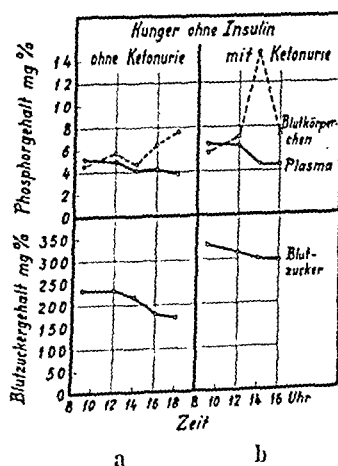


Abb. 71. Verteilung des Phosphors auf Blutkörperchen und Plasma im Hunger.

a Bei nicht ketonkörperbildendem Diabetes (Mittelwerte von 4 Fällen).  
b Bei einem Fall von Diabetes mit starker Ketonurie ohne Insulin.

eigentliche Wesen dieser Störung ist zur Zeit nicht näher bekannt.

Bei hochgradiger Ketonurie in Verbindung mit Praekoma wird die Phosphoranhäufung in den Blutkörperchen noch stärker. Abb. 72 b macht den Phosphorgehalt der Blutkörperchen bei einem Fall von bedrohlichem Diabetes ersichtlich, wo die Insulinbehandlung wegen praekomatösen Erbrechens um 12.10 Uhr eingeleitet werden musste. Im Gegensatz hierzu zeigt der Blutzuckergehalt, nach Hagedorn bestimmt, sowohl in den Blutkörperchen wie im Plasma nach der Insulingabe ein unmittelbares, starkes Sinken. Ob diese Senkung auf einem Verschwinden des Blutzuckers oder auf einer Blockierung wirksamer reduzierender Aldehydgruppen beruht, ist noch nicht geklärt; ebensowenig ist bekannt, in welcher Form der Phosphor in den Blutkörperchen gebunden wird (vgl. S. 286). Ausscheidungsdiagramme von diesem Fall findet man in Abb. 73, welche die  $\beta$ -Oxybuttersäureausscheidung während des Hungertages und das Verschwinden derselben unter dem Einfluss des Insulins in derselben Weise wie Abb. 72 ersichtlich macht.

Die folgende Krankengeschichte mit Ausscheidungsdiagramm illustriert den betreffenden Diabetesfall:

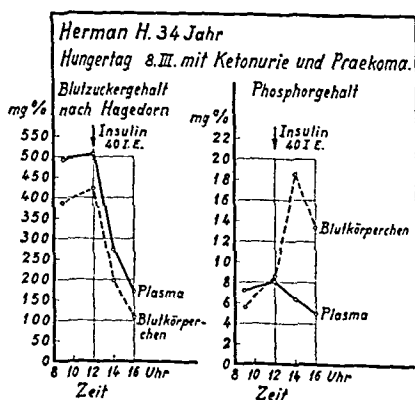


Abb. 72. Phosphor- und Blutzuckergehalt nach Hagedorn von Blutkörperchen und Plasma bei einem Fall von bedrohlichem Diabetes mit Praekoma. Insulin wurde um 12.10 Uhr gegeben. Man beachte die starke Senkung des Reduktionswerts bei steigendem Phosphorgehalt der Blutkörperchen.



Fall 18. Herman H., geb. 1907.

### Vater leidet an Diabetes.

Pat. war früher stets gesund. Im August 1933 zunehmender starker Durst, Schwächegefühl und Gewichtsabnahme (6 kg in 14 Tagen). Harnmenge 12—15 1/24 st. Nach 14 Tage wurde der Diabetes konstatiert, vierwöchige Krankenhausbehandlung. Bei der Entlassung strenge Diät und 32 + 32 I. E. Insulin. Im November 1934 Verschlimmerung mit Koma, von neuem stationäre Behandlung, bei der die Insulindosis auf 72 + 72 I. E. pro die erhöht wurde, auch etwas reichlichere Kost. Die Insulinmenge wurde nach und nach auf 40 + 40 I. E. herabgesetzt. — Bei der Beobachtung im März 1941: Länge 178 cm, Gewicht 69,3 kg. Pulzfrequenz 80, Blutdruck 155/80. Leber etwas vergrößert, 1 Querfinger breit unter dem Rippenbogen palpabel, innere Organe sonst o. B. Der Diabetestypus geht aus dem Ausscheidungsdiagramm (Abb. 73) hervor.

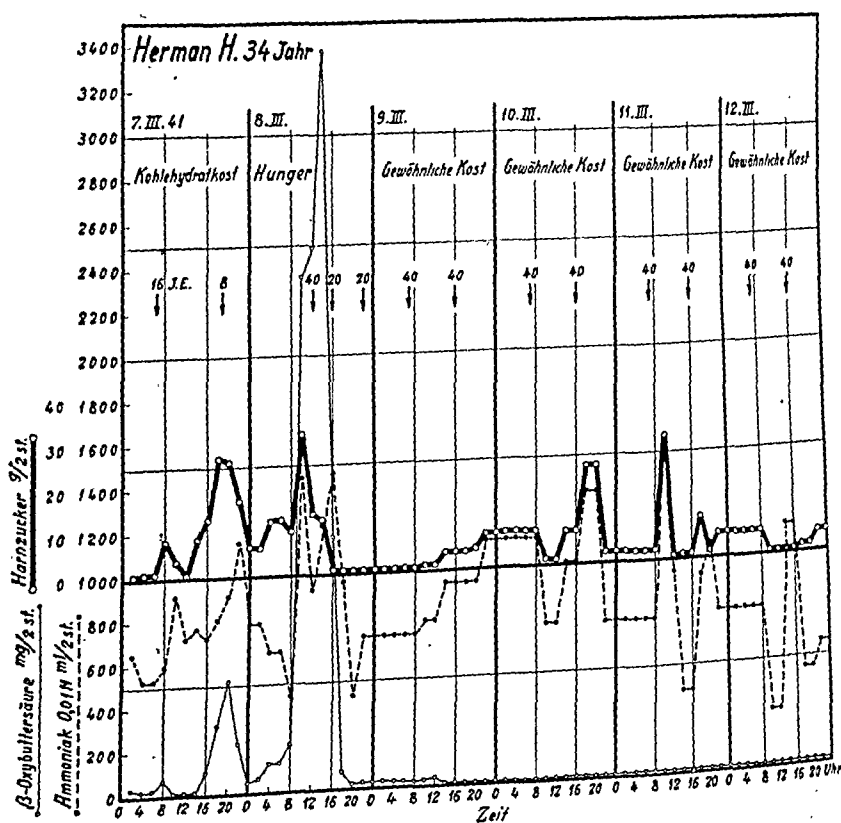


Abb. 73. Ausscheidungsdiagramm bei bedrohlichem Diabetes mit Prækoma.  
Abb. 72 vom Hungertage (8. III.).

## Chlor im Blut und Urin

Im Gegensatz zur Phosphorsäure beteiligt sich das Chlor nicht direkt am intermediären Kohlehydratstoffwechsel; jedenfalls ist die Wirkung desselben bisher nicht näher bekannt. Dagegen spielen die Chlorionen eine Rolle als Regulationsfaktor für das Säuren-Basengleichgewicht des Blutes (vgl. VII. Abschnitt).

Soweit das Chlor als Chlornatrium gebunden ist, besitzt dasselbe auch für das osmotische Gleichgewicht Bedeutung. Trotz Schwankungen des Blutzuckergehalts ist der Chlorgehalt der Blutkörperchen und des Plasmas ziemlich konstant (Abb. 74).

Ist der Chlorgehalt des Blutplasmas verhältnismässig konstant, so wechselt auf der anderen Seite die im Urin ausgeschiedene Chlormenge. Die wechselnde Chlorzufuhr mit der Nahrung wird durch die variierende Chlorauscheidung ohne weiteres ausgeglichen, so dass diese den Körper passierenden Chlormengen nichts mit der Rolle des Chlors bei der Verdauung sowie mit seiner Beteiligung an der Säuren-Basenregulation zu tun haben (vgl. S. 256). Es ist möglich, dass das Chlor-

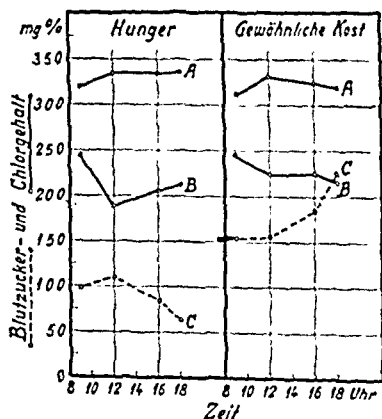


Abb. 74. Chlorgehalt des Blutplasmas (A) und der Blutkörperchen (B) sowie Veränderungen des reduzierenden Blutzuckers, nach Hagedorn bestimmt (C), bei einem Fall von Diabetes ohne Ketonurie, teils im Hunger, teils bei Nahrungszufuhr. Der Chlorgehalt wird von dem Schwanken des Blutzuckerspiegels nicht beeinflusst.

ausserdem bei den intermediären Stoffwechselprozessen irgendeine Rolle spielt, wenn auch darüber noch nichts bekannt ist. *Gerritzen* hat eine 24stundenperiodische Chlorausscheidung nachgewiesen, welche er in Beziehung zur rhythmischen Leberfunktion setzt. *Arborelius* hat diese 24stundenrhythmische Chlorausscheidung bei verschiedenen pathologischen Zuständen eingehender studiert und in derselben einen auch beim Fehlen diabetischer Stoffwechselveränderungen brauchbaren Indikator für endogene Rhythmusstörungen gefunden.

Bei zunehmender Azidose im Zusammenhang mit der Anhäufung von Ketonkörpern im Organismus verlassen Chlorionen die roten Blutkörperchen, was bei der Säuren-Basenregulation eine Rolle spielt.

### Auftreten von Intermediärprodukten beim Diabetes

Die intermediären Stoffwechselprodukte der Kohlehydrate lassen sich hinsichtlich ihres Bildung während der anoxydativen oder oxydativen Phase des Abbauprozesses (vgl. IV. Abschnitt) in Gruppen einteilen. Beim Diabetes, wo eine Störung im Kohlehydratabbau vorliegt, kann man daher erwarten, im Urin Intermediärprodukte wiederzufinden, welche verschiedene Stadien des Abbauverlaufs repräsentieren. Auf Grund der endogenen Rhythmik des Stoffwechsels findet dabei eine periodische Anhäufung gewisser Substanzen statt, welche sich im Urin durch mehr oder minder deutliche Ausscheidungswellen widerspiegelt. An Hand von Ausscheidungsdiagrammen lassen sich demnach Bildung und Verschwinden der Intermediärprodukte beim Diabetes bis zu einem gewissen Grade verfolgen, wodurch man ein Bild vom Verlauf der Stoffwechselstörung erhalten und in gewissen Fällen auch Rückschlüsse auf die Art derselben ziehen kann. Das Bild wird von einer systematischen Blutuntersuchung vervollständigt.

Für die genaue Analyse der ausgeschiedenen Substanzen ist es erforderlich, dass alle enzymatischen Prozesse unmittelbar

nach Entleerung des Harns abgebrochen werden, damit labilere Substanzen keine Veränderungen erleiden. Der Urin muss auch in kurzen Zeitabständen entleert werden und darf nicht zu lange in der Blase verweilen. Am einfachsten lässt sich die Reaktionshemmung durch Gefrieren des Urins unmittelbar nach der Miktion erzielen, und zwar mit Kohlensäureschnee bis zu sehr niedriger Temperatur ( $-80^{\circ}$ ).

Es folgen hier einige Studien über das Auftreten intermedärer Produkte beim Diabetes.

### Brenztraubensäure

Unter den Zwischenprodukten des Kohlehydratabbaus steht die Brenztraubensäure an bevorzugter Stelle (vgl. IV. Abschnitt). Auch beim Diabetes liegt der Brenztraubensäuregehalt des Blutes verhältnismässig konstant. Tab. 21 zeigt den höchsten beobachteten Brenztraubensäuregehalt des Blutes bei 75 Fällen von Diabetes während eines Hungertages ohne Insulin.

TAB. 21.

Höchster beobachteter Brenztraubensäuregehalt im Blut während eines Hungertages.

Brenztraubensäuregehalt mg %	Anzahl Fälle
1,26—1,50	2
1,51—1,75	10
1,76—2,00	12
2,01—2,25	17
2,26—2,50	20
2,51—2,75	5
2,76—3,00	7
3,01—3,25	1
3,26—3,75	1

Die Variationen des Brenztraubensäuregehalts des Blutes im Hunger werden aus der folgenden Tabelle 22 ersichtlich.

TAB. 22.  
Differenz zwischen höchstem und niedrigstem Brenztraubensäure-  
gehalt im Blut während eines Hungertages ohne Insulin.

Variationsbreite mg %	Anzahl Fälle
0,01—0,10	23
0,11—0,20	18
0,21—0,40	11
0,41—0,60	11
0,61—0,80	8
0,81—1,00	2
1,01—1,30	2

Der Brenztraubensäuregehalt des Blutes steigt in der Regel bei Kohlehydratzufuhr nicht, sondern weist oft eine Tendenz zum Sinken auf. In Tab. 23 findet man die höchsten beobachteten Brenztraubensäurewerte im Blut bei Kohlehydratzufuhr ohne Insulin bei 20 Fällen von Diabetes.

TAB. 23.  
Höchster beobachteter Brenztraubensäuregehalt im Blut bei  
Kohlehydratzufuhr ohne Insulin.

Brenztraubensäuregehalt mg %	Anzahl Fälle
0,76—1,00	1
1,01—1,50	1
1,51—2,00	6
2,01—2,50	5
2,51—3,00	6
3,01—3,50	1

Auch bei kohlehydratreicher Nahrung treten keine grösseren Variationen des Brenztraubensäuregehalts im Blute auf, was aus Tab. 24 hervorgeht.

Wenn auch der Brenztraubensäuregehalt des Blutes beim Diabetes oft verhältnismässig konstant ist, kann doch die im Urin ausgeschiedene Brenztraubensäuremenge bisweilen erhebliche Schwankungen zeigen. Abb. 75 zeigt das Schwanken

TAB. 24.

Differenz zwischen höchstem und niedrigstem Brenztraubensäuregehalt im Blut bei Kohlehydratzufuhr ohne Insulin.

Variationsbreite mg %	Anzahl Fälle
0,01—0,10	4
0,11—0,20	2
0,21—0,40	3
0,41—0,60	5
0,61—0,80	2
0,81—1,00	3
1,01—1,30	1

der Brenztraubensäureausscheidung bei verhältnismässig konstantem Brenztraubensäuregehalt des Blutes bei zwei Fällen von Diabetes. Bisweilen besteht eine markant 24stundenrhythmische Ausscheidung.

Die Ausscheidungsdiagramme veranschaulichen daher Bildung und Verschwinden der Brenztraubensäure im Organismus viel deutlicher als die Bestimmung der Brenztraubensäurefluktuationen im Blute.

Die Bedeutung der Kost für die Brenztraubensäureausscheidung beim Diabetes wird aus Tab. 25 ersichtlich.

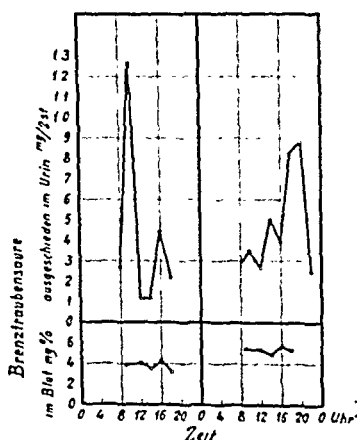


Abb. 75. Brenztraubensäuregehalt des Blutes und ausgeschiedene Brenztraubensäuremenge. NB. Erhebliche Schwankungen der ausgeschiedenen Brenztraubensäuremenge bei gleichbleibendem Brenztraubensäuregehalt des Blutes.

TAB. 25.

Ausscheidung von Brenztraubensäure, Zucker und  $\beta$ -Oxybuttersäure  
im Hunger und bei Kohlehydratzufuhr bei Diabetes  
mit und ohne Ketonurie.

Diabetestypus	Hunger			Kohlehydratkost		
	Ausscheidung 8—22 Uhr			Ausscheidung 8—22 Uhr		
	Brenztraubensäure mg	$\beta$ -Oxybuttersäure mg	Zucker g	Brenztraubensäure mg	$\beta$ -Oxybuttersäure mg	Zucker g
<i>Mit Ketonurie</i>						
Karl B. ....	95,8	5047	16,8	31,1	3024	86,6
Anders A. ....	36,4	423	6,1	53,6	1031	67,0
Tora A. ....	16,9	192	19,8	23,3	1128	51,4
Gustav W. ....	26,1	1194	16,7	30,5	1817	45,2
Arvid P. ....	28,2	307	3,6	27,1	406	28,6
<i>Ohne Ketonurie</i>						
Ingegärd K. ....	14,0	0	0	35,3	0	68,4
Birgitta S. ....	13,0	0	0	37,0	0	110,9
Ida B. ....	18,3	0	15,7	20,4	0	38,5
Arvid S. ....	10,5	0	0	11,1	0	0
Erik U. ....	14,6	0	0	36,7	0	43,3

Aus der Tabelle geht hervor, dass der Einfluss der Kohlehydratkost auf die Brenztraubensäureausscheidung nicht einheitlich ist: bei einem Teil der Fälle steigt diese bei Nahrungszufuhr, bei anderen sinkt sie und bei noch anderen besteht kein nennenswerter Unterschied zwischen den im Hunger und bei Kohlehydratkost ausgeschiedenen Brenztraubensäuremengen. Ein Parallelismus zwischen Brenztraubensäure- und  $\beta$ -Oxybuttersäureausscheidung liegt, wie man sieht, nicht vor.

### Zitronensäure

Die Bedeutung des Zitronensäurezyklus für den oxydativen Abbau der Kohlehydrate ist im IV. Abschnitt des näheren behandelt worden. Bei Störungen im Kohlehydratstoffwechsel kann die Ausscheidung der Intermediärsubstanzen einen gewissen Fingerzeig für die Beurteilung der Lokalisation der Stoffwechselstörung im Abbauprozess geben.

Zur ersten Orientierung bezüglich der Frage, ob der Zitronensäureumsatz beim Diabetes gestört ist, dient das Studium der Ausscheidung der Zitronensäure. Es ist noch nicht möglich, die biochemische Bedeutung einer gesteigerten Zitronensäureausscheidung zu beurteilen, da hierzu u. a. eine eingehende Analyse aller zum Zitronensäurezyklus gehörender Substanzen erforderlich wäre. Eine vermehrte Ausscheidung kann jedoch auf irgendeine Veränderung im Verlauf des Zitronensäureumsatzes und damit auf eine Störung der dehydrierenden Prozesse in dem oxydativen Abbau der Kohlehydrate hindeuten (vgl. IV. Abschnitt).

Tab. 26 macht die Ausscheidung von Zitronensäure, Zucker und  $\beta$ -Oxybuttersäure bei Diabetes mit und ohne Ketonurie ersichtlich.

TAB. 26.

Ausscheidung von Zitronensäure, Zucker und  $\beta$ -Oxybuttersäure im Hunger und bei Kohlehydratzufuhr bei Diabetes mit und ohne Ketonurie.

Diabetestypus	Hunger			Kohlehydratkost		
	Ausscheidung 8—22 Uhr			Ausscheidung 8—22 Uhr		
	Zitronensäure mg	$\beta$ -Oxybuttersäure mg	Zucker g	Zitronensäure mg	$\beta$ -Oxybuttersäure mg	Zucker g
<i>Mit Ketonurie</i>						
Gerda A. ....	532	5003	16,5	659	5238	83,7
Anna C. ....	609	2736	55,0	417	9436	103,5
Östen L. ....	418	295	4,7	482	116	63,8
<i>Ohne Ketonurie</i>						
Elmar D. ....	927	0	5,6	1042	0	32,0
Anna N. ....	815	0	2,8	939	0	31,8
Anna L. ....	787	0	0	712	0	0,16

Aus der Tabelle ergibt sich, dass Kohlehydratkost keine konstante Wirkung auf die Zitronensäureausscheidung ausübt, und dass keinerlei Parallelismus zwischen Zitronensäure- und  $\beta$ -Oxybuttersäureausscheidung besteht. Beim Diabetes ohne Ketonurie scheint eine vermehrte Ausscheidung von Zitronensäure vorzuliegen.



Die Zitronensäure lässt namentlich im Hunger eine mehr oder minder deutlich hervortretende periodische Ausscheidung erkennen. In Abb. 76 a und b findet man die Ausscheidung von Brenztraubensäure,  $\beta$ -Oxybuttersäure und Zitronensäure bei zwei Fällen von Diabetes, einem mit und einem ohne Ketonurie, im Hunger und bei Kohlehydratkost.

Bei dem ersten Fall mit Ketonurie (Abb. 76 a) wird die Zitronensäure im Hunger periodisch ausgeschieden, mit einem Maximum an demselben Zeitpunkt wie bei der Brenztraubensäure, dagegen nicht mit dem Ausscheidungsmaximum der  $\beta$ -Oxybuttersäure zusammenfallend. In diesem Falle geht die Zitro-

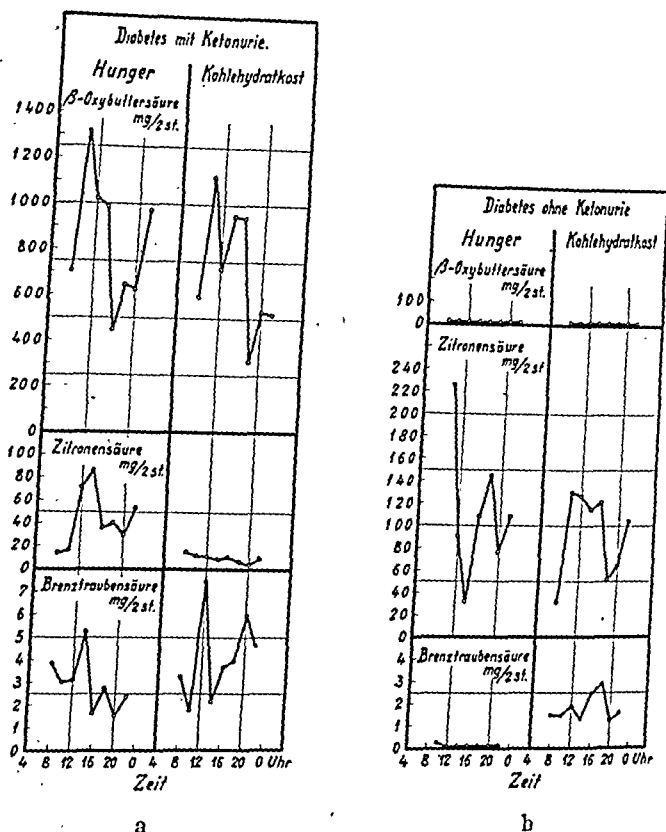


Abb. 76. Ausscheidung von Brenztraubensäure und Zitronensäure im Hunger- und bei Kohlenhydratnahrung.

a Diabetes mit Ketonurie. b Diabetes ohne Ketonurie.

Man beachte die geringe Zitronensäureausscheidung und kräftige Brenztraubensäureausscheidung in a bei Kohlenhydratzufuhr, dagegen aber die geringe Brenztraubensäureausscheidung bei starker Zitronensäureausscheidung in b im Hunger.

nensäureausscheidung bei Kohlehydratzufuhr merkbar zurück, wobei die Periodizität trotz einer markanteren rhythmischen Brenztraubensäureausscheidung verschwindet.

Bei dem zweiten Fall ohne Ketonurie (Abb. 76 b) ist die Zitronensäureausscheidung sowohl im Hunger wie bei Kohlehydratkost stärker. Hier ist die Brenztraubensäureausscheidung während des Hungertages ganz unerheblich.

Es liegt also bei diesen Fällen ein Unterschied zwischen Diabetes mit und ohne Ketonurie hinsichtlich der Zitronensäureausscheidung vor. Dies kann das Zeichen einer Störung im Zitronensäureumsatz und damit im dehydrierenden, oxydativen Kohlehydratabbau des Organismus sein. Diese Frage ist noch nicht geklärt.

## Pyridin

Die Dehydrierungsprozesse beim oxydativen Abbau der Kohlehydrate setzen die Mitwirkung dehydrierender Fermentsysteme voraus. Dabei spielt das Pyridin eine hervorragende Rolle; es ist als prosthetische Gruppe in denjenigen Codehydra-

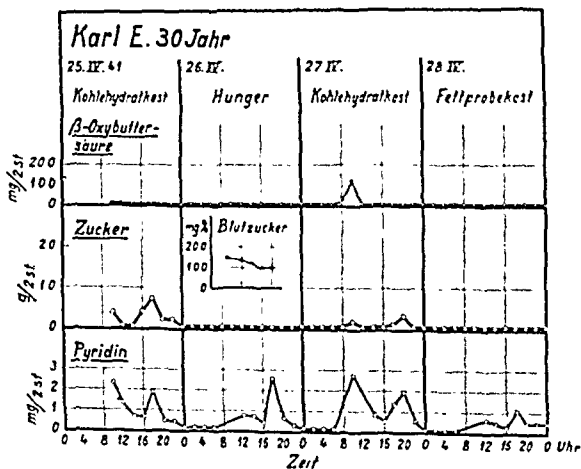


Abb. 77. Ausscheidung von Pyridin, Zucker und  $\beta$ -Oxybuttersäure im Hunger, bei Kohlenhydrat- und bei Fettkost ohne Insulin. Man beachte die 24stundensperiodische Pyridinausscheidung auch im Hunger mit zwei Maxima während des Tages. In der Nacht ist bei diesem Falle fast jegliche Pyridinausscheidung verschwunden. Kohlehydratkost bringt die Pyridinausscheidung zum Steigen, fettreiche Ernährung zum Sinken.

sen enthalten, welche sich am Kohlehydratabbau beteiligen. Das Studium der Pyridinausscheidung bei verschiedenen Formen von Diabetes besitzt daher Interesse.

Das Pyridin tritt nach *Field* und *Melnick* im Harn teils als freie Nikotinsäure auf, evtl. an Aminostickstoff oder Glykokoll gekoppelt, mit dreiwertigem Stickstoff im Pyridinkern, teils, und zwar zum grössten Teil, in methylierter Form als Trigonellin mit fünfwertigem Stickstoff im Kern. Letztere Fraktion stammt grösstenteils aus der Nahrung und besitzt bezüglich des endogenen Pyridinumsatzes gelegentlich der Wirkung der Dehydrasen im Organismus geringeres Interesse.

Abb. 77—80 zeigen die Ausscheidung von Pyridin mit dreiwertigem Kernstickstoff bei 4 Fällen von Diabetes.

Fall 19. Karl E., geb. 1911.

Kein Diabetes in der Familie.

Im Dezember 1938 zunehmendes Durstgefühl, nahm in 3 Monaten 18 kg ab (80—62 kg). Der Diabetes wurde im März 1939 konstatiert. Erhielt nie Insulin. Abb. 77 macht die Ausscheidung von Zucker,  $\beta$ -Oxybuttersäure und Pyridin während des Tages bei Hunger, Kohlehydrat- und Fettprobekost ersichtlich. Die Pyridinausscheidung lässt eine deutliche 24Stundenperiodizität erkennen und ist in der Nacht bei diesem Falle fast gänzlich verschwunden. Bei der Fettprobekost nimmt die Pyridinausscheidung ab.

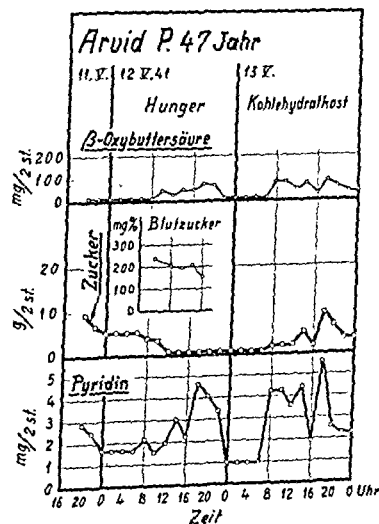


Abb. 78. Ausscheidungsdiagramm bei Diabetes mit starker Pyridinausscheidung, aber geringer Zuckerdiurese und  $\beta$ -Oxybutterausscheidung, ohne Insulin.

Fall 20. Arvid P., geb. 1891.

Ein Bruder und eine Schwester leiden an Diabetes.

Im August 1930 Durst und beginnende Abmagerung. Gleichzeitig wurde der Diabetes festgestellt. Strenge Diät, Pat. nahm in zwei Jahren von 82 bis 70 kg ab. Seit Frühjahr 1937 Herabsetzung des Sehvermögens, Abnahme des Gehörs und Ohrensausen, Alveolarpyorrhoe mit Ausfällen von Zähnen. Impotentia coeundi. Insulinbehandlung wurde 1939 wegen allgemeiner Verschlimmerung des Zustands eingeleitet. Abb. 78 zeigt die Pyridin-, Zucker- und  $\beta$ -Oxybut-

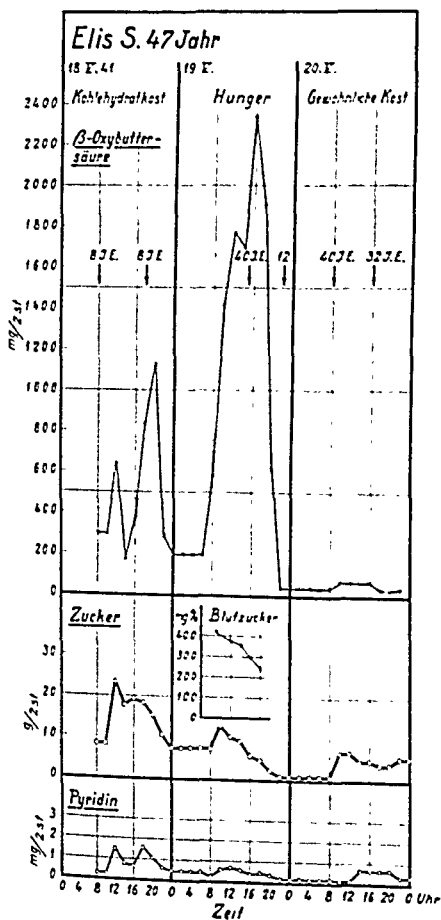


Abb. 79. Ausscheidungsdiagramm bei Diabetes mit starker Ketonurie und Praxkoma sowie beträchtlicher Zuckerkidiurese, aber relativ geringer Pyridinausscheidung.

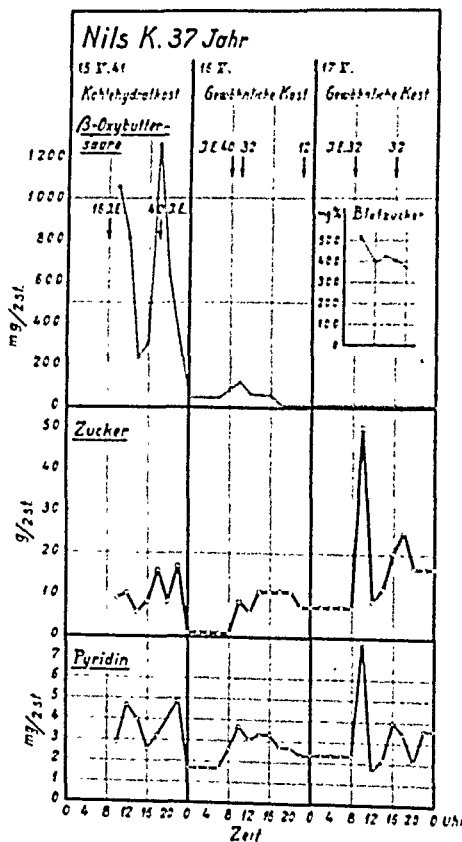


Abb. 80. Ausscheidungsdiagramm bei bedrohlichem Diabetes mit Insulin und Nahrungszufuhr. Starke Pyridinausscheidung sowohl bei Ketonurie als auch nach Verschwinden derselben infolge von Steigerung der Insulindosis. Man beachte in diesem Fall den Parallelismus zwischen Pyridin- und Zuckerausscheidung nach dem Aufhören der Ketonurie.

tersäureausscheidung bei Hunger und Kohlehydratkost. Aus den Diagrammen geht hervor, dass es sich trotz relativ geringfügiger Zuckerdiurese und Ketonurie um eine erhebliche Pyridinausscheidung handelt, welche auch bei diesem Falle eine merkbare 24Stundenperiodizität mit einem Minimum in der Nacht aufweist.

*Fall 21. Elis S., geb. 1894.*

Ein Bruder des Vaters hat Diabetes.

1920 wurde zufällig Zucker im Urin festgestellt. 1930 Magengeschwür. Strenge Diät, Verschlimmerung. Im September 1932 plötzlich quälendes Durstgefühl, nun auch Säuren im Urin. Strenge Diät. Nervöse Beschwerden mit Krämpfen und Kolikschmerzen. Insulinbehandlung wurde 1934 eingeleitet. In Abb. 79 findet man die Pyridin-, Zucker- und  $\beta$ -Oxybuttersäureausscheidung bei Hunger und Nahrungszufuhr mit Insulin. Trotz Zuckerdiurese, erheblicher Ketonurie und Prækoma bei Verminderung der Insulindosis lag hier nur eine geringfügige Pyridinausscheidung vor, auch in diesem Falle 21stundenperiodisch mit einem Ausscheidungsminimum in der Nacht.

*Fall 22. Nils K., geb. 1904.*

Keine erbliche Veranlagung für Diabetes.

Im November 1939 plötzlich einsetzendes Durstgefühl, Pat. nahm in einer Woche 7 kg ab. Es wurde da der Diabetes konstatiert und eine Insulinbehandlung eingeleitet. Abb. 80 macht eine recht starke Pyridinausscheidung ersichtlich, die teilweise der Zuckerdiurese folgt, aber anscheinend von der bei gesteigerter Insulindosis völlig verschwindenden  $\beta$ -Oxybuttersäureausscheidung ganz unabhängig ist.

Die Bedeutung einer vermehrten Pyridinausscheidung beim Diabetes ist noch unbekannt. Dieselbe folgt der Zuckerausscheidung nicht obligat und hängt wahrscheinlich nicht von der Ketonurie und Ketonkörperbildung ab. Auffallend ist ihr 24stundenperiodischer Verlauf, oft mit zwei Maxima am Tage, einem am Vor- und einem am Nachmittag, sowie die Abnahme während der Nacht. Wahrscheinlich spiegelt die Pyridinausscheidung den endogenen Rhythmus mit seinen wechselnden Phasen wider und ändert sich mit den Assimilations- und Dissimilationsperioden des Organismus. Abb. 81 zeigt Mittelwertkurven der Pyridin- und Brenztraubensäureausscheidung beim ketonkörperbildenden Diabetes im Hunger, bei Kohlehydrat- und Fettkost, Abb. 82 entsprechende Kurven bei Diabetes ohne Ketonkörperbildung. Auffallend ist, dass die Pyridinausscheidung in beiden Fällen im Hunger stark ist und einen periodischen Verlauf mit Maxima um 10 und 18 Uhr sowie einem

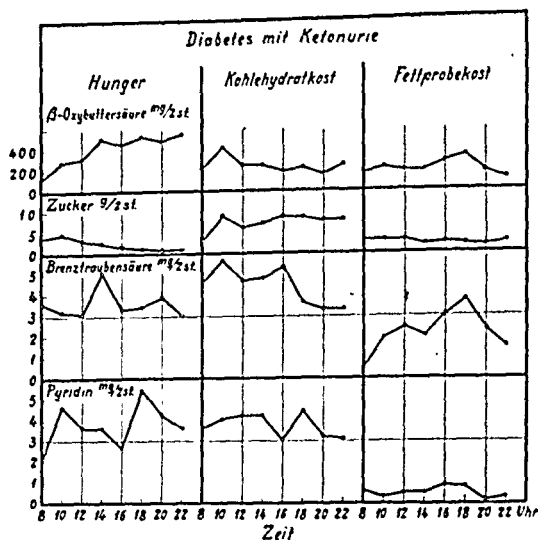


Abb. 81. Ausscheidungsdiagramm bei Diabetes mit Ketonurie im Hunger sowie bei kohlehydrat- und fettreicher Kost (Mittelwertkurven von 22 Fällen ohne Insulin). Zu beachten ist die starke, periodische Pyridinausscheidung im Hunger mit einem Brenztraubensäuremaximum abwechselnd mit den Maxima des Pyridins. Die Pyridinausscheidung wird bei fettreicher Kost geringer.  $\beta$ -Oxybuttersäureausscheidung im Hunger am stärksten.

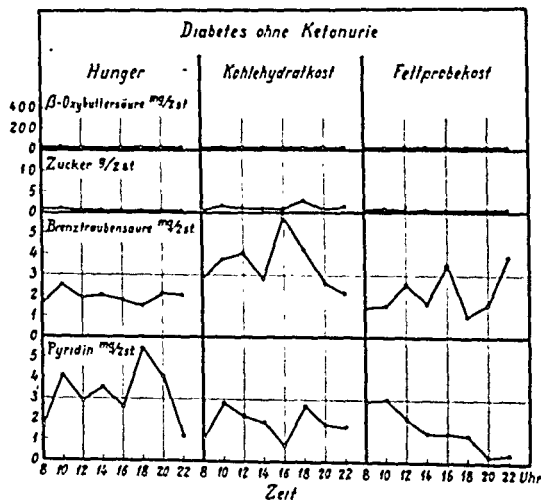


Abb. 82. Ausscheidungsdiagramm bei Diabetes ohne Ketonurie (Mittelwertkurven von 25 Fällen ohne Insulin). Pyridinausscheidung im Hunger am stärksten, bei Kohlehydratkost geringer und bei fettreicher Ernährung abnehmend. Gestiegene Brenztraubensäureausscheidung bei Kohlehydratkost.

Tagesminimum um 16 Uhr erkennen lässt. Fettkost scheint die Pyridinausscheidung erheblich herabzusetzen, namentlich bei ketonkörperbildenden Diabetesformen.

Nahrungszufuhr macht die Pyridinausscheidung nicht stärker als im Hunger, und bei Fettkost ergibt sich auch hier eine merkbare Abnahme derselben. Es hat daher den Anschein, als ob eine fettreiche Ernährung den Körper speziell gegen Pyridinverluste schützt und damit womöglich auch die dehydrierenden Fermentsysteme desselben vor dem Zerfall bewahrt. Die physiologisch-chemische Bedeutung der Pyridinausscheidung ist allerdings noch ungeklärt.

### Die Cholinausscheidung beim Diabetes

Wenn beim ketonkörperbildenden Diabetes die Insulinwirkung nachlässt, dann nimmt die Ketonkörperbildung zu. Dabei werden gleichlaufend mit der vermehrten  $\beta$ -Oxybuttersäure auch Phosphorsäure und Cholin in gesteigerten Mengen ausgeschieden (vgl. S. 297). Wird die Insulinbehandlung eingeleitet, so geht die Phosphorsäure-, Cholin- und  $\beta$ -Oxybuttersäureausscheidung wieder zurück (Abb. 68).

Dieser Sachverhalt deutet darauf hin, dass die  $\beta$ -Oxybuttersäurebildung wahrscheinlich mit einer Störung im Phosphatidumsatz zusammenhängt. Man hat den Eindruck, dass das Fett, um in normaler Weise verbrannt werden zu können wie die Kohlehydrate, phosphoryliert werden und mit einer Stickstoffbase, z. B. Cholin, gekoppelt in einen Phosphatidkomplex eingehen muss. Erst dann scheint der normale oxydative Abbau der Fettsäuren vor sich zu gehen. Das Cholin spielt möglicherweise im Fettstoffwechsel eine ähnliche Rolle wie die Cozymase beim Kohlehydratabbau, aber hierüber ist noch nichts bekannt. Die Herabsetzung der Cholin-, Phosphorsäure- und  $\beta$ -Oxybuttersäureausscheidung durch Insulin (vgl. Abb. 68 S. 296) deutet darauf hin, dass das Insulin irgendwie in den Phosphatidumsatz eingreift, entweder im Sinne einer Steigerung der Phosphatidsynthese oder den Zerfall der Phosphatide hemmend. Der erhöhte Chylomikrongehalt des Blutplasmas

TAB. 27.

Ausgeschiedene Cholin- und  $\beta$ -Oxybuttersäuremengen bei Diabetes mit und ohne Ketonkörperbildung im Hunger ohne Insulin.

Typus	Cholin		$\beta$ -Oxybuttersäure		$\beta$ -Oxybuttersäure Cholin
	mg	Millimol	mg	Millimol	
<i>Mit Ketonkörperbildung.</i>					
Fall 1 .....	793	6,5	4800	46,10	6,9
» 2 .....	600	5,0	4912	47,23	9,4
» 3 .....	458	3,8	3189	31,66	8,3
» 4 .....	424	3,5	3610	34,70	9,9
» 5 .....	348	2,9	810	7,78	2,7
» 6 .....	334	2,8	985	9,47	3,3
» 7 .....	321	2,7	2420	23,27	8,6
» 8 .....	250	2,1	1199	11,53	5,5
» 9 .....	214	1,8	725	9,69	3,5
» 10 .....	146	1,2	5537	53,24	44,4
» 11 .....	127	1,0	1217	11,70	11,7
» 12 .....	120	1,0	195	18,75	18,8
» 13 .....	77	0,6	245	2,36	3,6
<i>Ohne Ketonkörperbildung.</i>					
Fall 1 .....	75	0,62	0	0	—
» 2 .....	10	0,08	0	0	—
» 3 .....	54	0,45	0	0	—
» 4 .....	101	0,83	0	0	—

unter dem Einfluss des Insulins (vgl. S. 33) kann vielleicht das Zeichen einer Phosphatidsynthese sein, aber diese Frage ist noch nicht geklärt. Ein ziemlich konstantes Grössenverhältnis zwischen ausgeschiedenem Cholin und  $\beta$ -Oxybuttersäure bei Ausscheidung grösserer Cholinmengen, wie es aus Tab. 27 ersichtlich wird, ist ein Hinweis auf eine gewisse Beziehung zwischen Cholin- und  $\beta$ -Oxybuttersäureausscheidung. Wenn es sich um kleinere Mengen Cholin handelt, variiert der Quotient  $\frac{\beta\text{-Oxybuttersäure}}{\text{Cholin}}$  mehr. Wahrscheinlich interferieren hier andere Prozesse, wodurch der wechselnde Ausscheidungsquotient zustande kommt. Liegt keine Ketonurie vor, dann ist die Cholinausscheidung sehr gering (Tab. 27).



Die Cholinausscheidung ist beim Ketonkörperbildenden Diabetes im Hunger am stärksten. Tab. 28 zeigt die Mittelwerte der Cholinausscheidung 6—20 Uhr bei 52 Fällen von Diabetes, 42 mit und 10 ohne Ketonurie (korrespondierende Untersuchung an demselben Patienten im Hunger, bei Kohlehydratkost und gewöhnlicher Kost).

TAB. 28.

Während des Tages im Hunger und bei Nahrungszufuhr ausgeschiedene Cholinmengen bei Fällen von Diabetes mit und ohne Ketonkörperbildung.

Typus		Ohne Insulin		Mit Insulin	
		Cholin mg/14 st	$\beta$ -Oxybuttersäure mg/14 st	Cholin mg/14 st	$\beta$ -Oxybuttersäure mg/14 st
mit Ketonurie A u. 0	Hunger .....	351	2296		
	Kohlehydratkost..	250	1270	70	107
	gewöhnliche Kost..	76	284	46	103
ohne Ketonurie B	Hunger .....	60	0		
	Kohlehydratkost..	143	0	94	0
	gewöhnliche Kost..	173	0	45	0

Wird Nahrung zugeführt, so sinkt die Cholinausscheidung beim Diabetes mit Ketonurie auch ohne Insulin. Besteht dagegen keine Ketonurie, dann ist die Cholinausscheidung im Hunger am geringsten, steigt aber bei Nahrungszufuhr. Hinsichtlich der Cholinausscheidung scheint daher ein grundsätzlicher Unterschied zwischen Diabetesformen mit und ohne Ketonurie vorzuliegen.

### Alkoholbildung beim Diabetes

Liegt beim Diabetes eine Störung im oxydativen Abbau der Kohlehydrate vor, so kann es zu einer Anhäufung von anaeroben Abbauprodukten kommen. Besonderes Interesse in dieser Beziehung besitzt das Auftreten des Äthylalkohols, dessen in grösserer Menge deletäre Wirkungen auf Gewebe und Organe

ja zur Genüge bekannt sind. Schon normalerweise enthalten Blut und Urin kleine Mengen von Äthylalkohol, das Blut etwa 10—50 mg%. Bei gewissen Fällen von Diabetes lässt sich Alkohol in erheblich grösseren Mengen nachweisen. Die bei Anaerobie auftretende Alkoholbildung ist in vielen Fällen auffallend periodisch, mit vorübergehenden Steigerungen des Blutalkohols und Ausscheidungswellen im Urin auch bei Hunger.

Der bei Störungen im Zwischenstoffwechsel auftretende Alkohol erscheint im Blute teilweise mehr oder weniger fest an Eiweiss gebunden, womöglich als Substrat an sein Fermentprotein gekoppelt. Man erhält dabei den Alkohol in freier Form

TAB. 29.  
Alkohol im Blut bei Diabetes.

Diabetestypus	Blut mg %										Urin	
	Alkohol					Zucker					Ausgeschiedene Menge 8—20 Uhr	
	Uhr					Uhr					Zucker g.	β-Oxybuttersäure mg
	9	12	14	16	18	9	12	14	16	18		
<i>Mit Alkoholbildung</i>												
Sperling, G., Hunger .....	134	231	130	158	128	193	209	171	138	141	0	0
Gunnar O., Hunger .....	41	164	0	40	82	126	98	92	81	86	7	0
Gustaf L., Hunger .....	105	409	53	137	120	268	237	230	190	198	19.7	210
Gewöhnl. Kost..	63	32	84	179	44	299	372	392	397	435	130	92
Lars Ö., Kohlehydratkost	118	197	114	104	123	277	235	331	212	325	106	2540
Gewöhnl. Kost + Insulin..	49	63	18	29	45	325	390	428	546	485	209	42
Ingvar Ö., Gewöhnl. Kost + Insulin .....	41	410	492	369	328	193	214	222	161	199	150	0
<i>Ohne Alkoholbil- dung</i>												
Nestor H., Hunger	42	21	33	21	69	258	233	247	226	205	7	198
Erik K., Hunger..	95	32	49	0	54	231	205	186	165	139	3	245
Ada H., Hunger..	55	89	102	27	78	444	353	369	346	372	54	3503
Anna N., Hunger	32	88	36	46	10	221	186	155	148	144	3	0
Rune O., Hunger	17	44	72	26	54	400	338	346	392	358	37	4549

durch die Hydrolyse, welche bei Alkoholbestimmungen in saurer Lösung spontan erfolgt. Dies ist bei der Friedeman-Klaas-schen Bestimmungsmethode (S. 432) der Fall, aber nicht bei der Widmarkschen, weshalb diese mitunter erheblich niedrigere Werte liefert.

Tab. 29 zeigt die Fluktuationen des Alkoholgehalts des Blutes während des Tages bei zehn Fällen von Diabetes, fünf mit und fünf ohne Alkoholbildung. Auch Blutzuckergehalt nach Hagedorn an denselben Zeitpunkten sowie die im Laufe des Tages ausgeschiedenen Mengen von Zucker und  $\beta$ -Oxybuttersäure sind angegeben. Aus der Tabelle ergibt sich, dass bei alkoholbildenden Diabetesformen im Hunger ein deutliches Alkoholmaximum um 12 Uhr auftritt, das bei Nahrungszufuhr mehr oder minder stark verschoben werden kann. Irgendeine Übereinstimmung zwischen Höhe des Blutzuckerwerts und Alkoholgehalt des Blutes liegt nicht vor. Die Alkoholbildung erfolgt unabhängig von Zuckerdiurese und Ketonurie.

Die Alkoholausscheidung im Urin folgt teilweise dem erhöhten Alkoholgehalt des Blutes. Abb. 83 macht den Blutalkohol

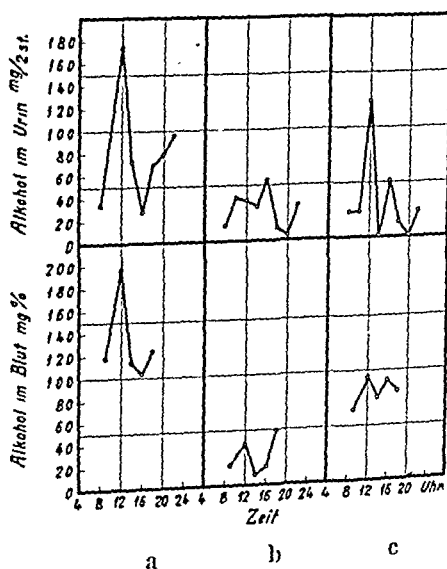


Abb. 83. Alkoholgehalt des Blutes und im Urin in zwei Stunden ausgeschiedene Alkoholmenge bei Diabetes.

- a Lars O., Kohlehydratprobekost. Insulin um 17 Uhr.  
 b Erik P., Hunger ohne Insulin.  
 c Rune O., gewöhnliche Kost mit Insulin: um 6 und 18 Uhr.

sowie die entsprechende pro Zweistundenintervall ausgeschiedene Alkoholmenge ersichtlich. Die Zunahme des Blutalkohols geht mit einer gesteigerten Alkoholausscheidung Hand in Hand, aber der Alkohol im Urin kann wie die Brenztraubensäure auch bei relativ gleichbleibendem Alkoholgehalt des Blutes von Stunde zu Stunde wechseln (Abb. 83 c).

Die der Alkoholausscheidung entsprechende Zucker- und  $\beta$ -Oxybuttersäureausscheidung geht aus Abb. 84 a, b und c hervor (derselbe Patient an den gleichen Tagen).

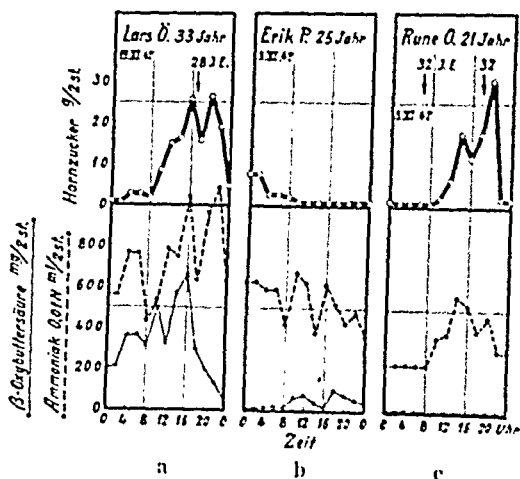


Abb. 84. Ausscheidungsdiagramme, die Zucker-Ammoniak- und  $\beta$ -Oxybuttersäuremenge im Urin entsprechend der Alkoholausscheidung in Abb. 83 zeigend.

Diese Ausscheidungsdiagramme machen ersichtlich, dass die stärkste Alkoholausscheidung nicht mit dem Maximum der Zuckerdiurese zusammenfällt. Alkoholbildung und Zuckerdiurese scheinen eher in einem gewissen gegensätzlichen Verhältnis zueinander zu stehen.

Da die Umsetzung der Zitronensäure einem oxydativen Kohlehydratabbauprozess entspricht und der Äthylalkohol ein anaerobes Abbauprodukt ist, besitzt ein Vergleich der Zitronensäureausscheidung mit der gleichzeitigen Ausscheidung von Alkohol nicht geringes Interesse. In Abb. 85 findet man die Zucker-, Zitronensäure- und Alkoholausscheidung bei Diabetes mit und ohne Ketonurie, teils im Hunger und teils bei

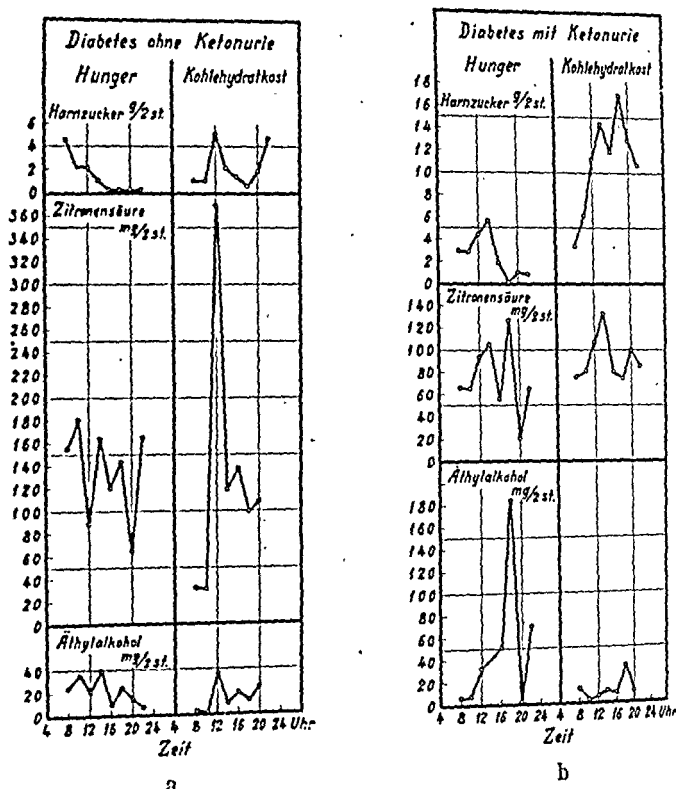


Abb. 85. Ausscheidung von Alkohol, Zitronensäure und Zucker im Hunger und bei Kohlehydratkost ohne Insulin bei zwei Fällen von Diabetes.

a Diabetes ohne Ketonurie.

b Diabetes mit Ketonurie.  $\beta$ -Oxybuttersäureausscheidung 6–22 Uhr im Hunger 5612 mg, bei Kohlehydratkost 6198 mg.

Kohlehydratkost. Die Alkoholausscheidung steigt bei kohlehydratreicher Nahrung nicht, sondern lässt im Gegenteil bei gewissen Fällen eine deutliche Abnahme erkennen, namentlich bei Diabetes mit Ketonurie. Dies deutet auf eine gesteigerte oxydative Umsetzung infolge der Zufuhr von Kohlehydraten hin. Dagegen nimmt die Zuckerdiurese zu.

Bei Diabetes ohne Ketonurie findet man oft eine erhöhte Zitronensäureausscheidung. Auch diese weist einen periodischen Wechsel auf, mitunter gleichlaufend mit den 24-Stundenvariationen der Alkoholausscheidung. Es ist daher wahrscheinlich, dass zwischen der Anaerobie im Organismus und dem Zitronensäureumsatz ein gewisser Zusammenhang besteht, aber diese Frage ist noch nicht spruchreif.

## Die klinische Analyse des diabetischen Zustands

Aus dem Vorstehenden wird ersichtlich, dass ein einfaches Studium des Reduktionsvermögens des Blutes als Ausdruck des Zuckergehalts desselben keine Richtschnur für die Beurteilung eines Diabetesfalls abgeben kann und ebensowenig einen sicheren Eindruck von den Kohlehydraten im Blut zu vermitteln vermag. Beim Diabetes, wo der Kohlehydratabbau gestört ist, weiss man überhaupt nicht, was man mit den üblichen Blutzuckerbestimmungsmethoden eigentlich bestimmt. In der laufenden klinischen Routinearbeit bietet eine genauere physiologisch-chemische Analyse der Kohlehydrate des Blutes Schwierigkeiten, und infolgedessen macht sich beim Diabetes in bezug auf die Bedeutung des Blutzuckerwerts eine sehr grosse Unsicherheit geltend. Für eine rationelle Diabetesstherapie ist aus diesem Grunde das Studium des Blutzuckerreduktionswerts eine sehr unvollkommene Hilfe. Man muss sich daher auf anderen Wegen Klarheit über das Wesen der Stoffwechselveränderung zu verschaffen suchen.

In vielen Beziehungen unterscheiden sich, wie oben beschrieben, die ketonkörperbildenden Diabetesformen von den nicht ketonkörperbildenden. Eine Gruppierung der einzelnen Diabetesfälle mit Rücksicht auf das Vorkommen von Ketonurie gewährt daher eine erste Möglichkeit zu einem vertieften Studium des Diabetesproblems. In vielen Fällen nimmt die Ketonkörperbildung bei sinkenden Blutzuckerwerten zu (S. 280). Der Hyperglykämie kann bei diesen Fällen eine kompensatorische Bedeutung zugeschrieben werden, im Sinne einer Beschränkung der Ketonkörperbildung, indem sie mit einer Besserung des Fett-Lipoidstoffwechsels und einer Verminderung der Cholinausscheidung und Ketonurie einhergeht (vgl. Tab. 28 S. 318). Der deletäre Einfluss der Unterernährung beim Diabetes geht u. a. aus der Tatsache hervor, dass auch die Pyridinausscheidung im Hunger trotz Verschwindens der Zuckerdiurese hoch ist. Dies gilt ebenso für Diabetesformen mit wie ohne Ketonurie. Wenn die vermehrte Pyridinausscheidung der Ausdruck eines Dehydrasezerfalls im Organismus (vgl. S.

172) ist, so muss dies bedeuten, dass Nahrungsmangel mit sinkenden Blutzuckerwerten eine mehr oder minder deutlich hervortretende Schädigung der dehydrierenden Fermentsysteme des Körpers zur Folge hat. Dadurch kommt es zu Störungen in den biologischen Oxydationsprozessen mit Veränderungen des Kohlehydratstoffwechsels, was sich u. a. durch eine gesteigerte Ketonkörperbildung im Hunger bemerkbar macht.

Die bei ketonkörperbildendem Diabetes im Hunger gesteigerte Cholinausscheidung ist ebenfalls ein Hinweis auf die Schädigung, welche dem Organismus durch den Nahrungsmangel zugefügt wird, auch wenn die Zuckerausscheidung verschwindet.

Ein anderer Exponent der Stoffwechselstörung ist das Auftreten von anaeroben Abbauprodukten in grösseren Mengen. Hierzu gehört der *Äthylalkohol*, der bei gewissen Diabetesformen auch im Hunger periodisch auftritt (vgl. S. 318). Derselbe nimmt bei Kohlehydratzufuhr nicht nennenswert zu. Ein erhöhter Blutzuckerwert kann der Ausdruck einer mit *Anaerobie* verbundenen Störung im Kohlehydratumsatz sein, aber der Alkoholgehalt des Blutes ist nicht obligat an die Höhe des Blutzuckerreduktionswerts gebunden (vgl. Tab. 28 S. 319). Möglicherweise häufen sich noch andere anaerobe Abbauprodukte mit bisher unbekannter Wirkung an, welche den Reduktionswert des Blutes erhöhen, aber diese Frage ist noch ungelöst.

Die wirksamen Faktoren der Blutzuckerregulation sind von den physiologisch-chemischen Vorgängen im Organismus abhängig. Unsere Kenntnisse über die Biochemie der Blutzuckerregulation sind allerdings noch sehr lückenhaft. Trifft die Stoffwechselstörung hauptsächlich die alimentäre Blutzuckerregulation, so machen sich die Wirkungen am meisten bei Nahrungsbelastung, weniger im Hunger, bemerkbar. Zeichen hierfür sind u. a. das Verschwinden der Zuckerdiurese bei sinkendem Blutzuckerspiegel, das Fehlen von Ketonurie und niedrige Cholinausscheidung bei Nahrungsentziehung. Wenn Nahrung zugeführt wird, steigt der Blutzuckerwert, und die Zuckerdiurese nimmt zu.

Spielt sich im Gegensatz hierzu die Stoffwechselstörung im

basalen Regulationsmechanismus ab, so zeigt sie sich sowohl im Hunger wie bei Nahrungsbelastung. Im Hunger tritt eine erhöhte Ketonurie, Phosphor- und Cholinausscheidung bei sinkenden Blutzuckerwerten auf, bei Nahrungszufuhr mit steigendem Blutzuckergehalt eine vermehrte Zuckerdiurese bei abnehmender Ketonurie. Dies kann auf eine Störung im Phosphorylierungsprozess hindeuten, welche im Hunger stärker wird.

Eine systematische Analyse von Ausscheidungsdiagrammen gewährt hierdurch eine Möglichkeit, den Charakter der Stoffwechselstörung genauer zu studieren, und damit eine bessere Richtschnur für die Beurteilung des Diabetesfalls als die Untersuchung des Blutzuckerreduktionswert, die bisher in grossem Umfang beim klinischen Diabetesstudium massgebend gewesen ist, aber nichts über das Wesen des Diabetesfalls sagt.

Von diesem Gesichtspunkt ausgehend habe ich ein klinisches Diabetesmaterial, welches aus über 1900 Diabetesfällen von verschiedenen Typen besteht, näher analysiert und mit systematischen Ausscheidungsdiagrammen an bestimmten Beobachtungstagen verfolgt.

Eine vollständige Untersuchungsserie enthält fünf Probetage (24 Stunden):

1. Kohlehydratprobekost,
2. Hunger,
3. Kohlehydratprobekost,
4. Fettprobekost,
5. Kohlehydratprobekost;

dann Einstellung des Kranken auf eine gemischte Normalkost oder diejenigen Kostbeschränkungen, welche bei dem speziellen Fall erforderlich sein können.

An den Probetagen ist das Kostregime folgendes:

*Kohlehydratprobekost.*

- 8 Uhr: 50 g Weissbrot, 10 g Butter, 20 g Kalbfleisch (auf Butterbrot), 20 g Magerkäse + Tee oder Kaffee ohne Zucker und Sahne.
- 12 Uhr: 30 g Weissbrot, 5 g Butter, 1 gekochtes Ei, 200 ccm Milch + Tee oder Kaffee.



16 Uhr: wie 8 Uhr.

20 Uhr: wie 12 Uhr.

### *Fettprobekost.*

8 Uhr: 10 g Weissbrot, 25 g Butter, 20 g Kalbfleisch, 20 g Magerkäse + Tee oder Kaffee ohne Zucker und Sahne.

12 Uhr: 10 g Brot, 20 g Butter, 1 gekochtes Ei, 200 ccm Milch + Tee oder Kaffee ohne Zucker und Sahne.

16 Uhr: wie 8 Uhr.

20 Uhr: wie 12 Uhr.

Dazu Wasser oder Mineralwasser nach Belieben gegen den Durst.

Am Hungertage darf der Kranke von 20 Uhr am vorangehenden Abend bis 8 Uhr am nächsten Morgen keinerlei Nahrung zu sich nehmen, weshalb die Hungerperiode 36 Stunden umfasst; an diesem Tage wird nur Flüssigkeit gegen den Durst zugeführt, evtl. Tee oder Kaffee ohne Zucker und Sahne.

Während dieser Probetage wird die Urinausscheidung in gleichmässigen Zweistundenintervallen von 6 Uhr bis 22 Uhr sowie alle vier Stunden nachts verfolgt. In jeder Harnportion werden routinemässig bestimmt: Titrationsazidität des Urins, pH-Wert (elektrometrisch mit Chinhydronelektrode und gesättigter Kalomel-Kaliumchloridelektrode), im Zeitintervall ausgetriggerte Ammoniakmenge (Formoltitrierung), Gesamtazidität, Gesamtazeton,  $\beta$ -Oxybuttersäure und Zucker. Während der Probetage, an welchen der Patient zu Bett liegt, um unnötige Muskelarbeit zu eliminieren, werden auch die Variationen der Körpertemperatur alle zwei Stunden sowie die Veränderungen des Körpergewichts von Tag zu Tag kontrolliert.

Die Ausscheidungsdiagramme für Zucker, Ammoniak und  $\beta$ -Oxybuttersäure werden graphisch aufgezeichnet und bilden die Grundlage für die weitere Behandlung des Diabetesfalls.

Hat der Kranke Insulinbehandlung erhalten, so wird die Insulindosis während einer Serie von Kohlehydratprobetagen Schritt für Schritt herabgesetzt, um nach Möglichkeit zu einem insulinfreien Hungertage zu kommen. Hierdurch kann man

Klarheit über die Ketonkörperbildung und den Schweregrad des Diabetesfalls erhalten. Bei den schweren Diabetesfällen ist es nicht möglich, einen insulinfreien Hungertag zu erreichen, da die  $\beta$ -Oxybuttersäurebildung bei ungenügender Ammoniakbildung und sinkendem Ammoniak- $\beta$ -Oxybuttersäurequotient zu stark wird. Wenn dabei der pH-Wert des Urins sinkt, entwickelt sich ein Praekoma, welches bald in ein Koma übergehen kann, falls nicht wieder Insulin eingesetzt oder die Insulindosis gesteigert wird. Bei weniger schweren Diabetesfällen kann das Insulin dagegen ohne Schwierigkeiten einen oder mehrere Tage ausgesetzt werden, zum Zweck eines eingehenderen Studiums der Stoffwechselstörung.

Die Ausscheidungsdiagramme lassen drei bestimmt zu unterscheidende Diabetestypen erkennen, welche der Einfachheit halber als Diabetes vom Typus A, Typus B und Typus O bezeichnet werden. Der letzte Typus entspricht einem bedrohlichen Diabetes, bei dem das Insulin nicht einmal 24 Stunden lang entbehrlich ist (0 insulinfreier Tag), ohne dass sich ein Praekoma oder Koma entwickelt. Hier liegt eine vitale Indikation zur Insulinbehandlung vor. Das Weglassen auch nur einer einzigen Insulindosis kann bei diesen Fällen infolge der starken und raschen Ketonkörperbildung dem Kranken zum Verhängnis werden.

## Diabetes vom Typus A

*Dieser Diabetestyp wird dadurch gekennzeichnet, dass im Hunger oder bei Nahrungsentziehung gleichzeitig mit der Abnahme oder dem Aufhören der Zuckerdiurese eine Ketonkörperbildung mit Ketonurie einsetzt. Der Blutzuckerwert kann dabei normal oder erhöht sein. Bei vermehrter Kohlehydratzufuhr steigt der Blutzuckerwert, wobei die Zuckerdiurese zunimmt. Gleichzeitig wird die Ketonurie geringer oder verschwindet ganz.*

In leichteren Fällen ist die Ketosäurenbildung beim A-Diabetes eine mässige und wird von einer Kompensationshyperglykämie unterdrückt. Dadurch, dass die Ammoniakbildung

hinreichend ist, bleibt der Ammoniak- $\beta$ -Oxybuttersäurequotient grösser als 1, was zur Folge hat, dass der pH-Wert des Urins nicht auf kritische Werte sinkt. Trotz der Ketosäurenbildung kommt es daher auch im Hunger nicht zu einer Nierenschädigung oder zu einem Prækoma.

Fall 23 ist ein Beispiel für einen A-Diabetes von dieser Art:

Fall 23. Matilda C., geb. 1873.

Keine erbliche Veranlagung für Diabetes. In den Jahren 1915–1923 Gallensteinbeschwerden; nie ikterisch. In den letzten Jahren beschwerdefrei, nicht operiert. Im März 1933 starkes Durstgefühl, Müdigkeit und Abmagerung (nahm in einem Monat 7 kg ab). Es wurde der Diabetes konstatiert und der Patientin strenge Diät verordnet, wobei der Zucker verschwand. Seit dieser Zeit Herzbeschwerden mit Oppressionsgefühl und zeitweise präkordialen Schmerzen sowie Knöchelödemen. 26. I. 1938 Länge 159 cm, Gewicht 78,9 kg. Blutdruck 190/100. Herz: Töne dumpf, rein, keine Geräusche. Keine Arrhythmie. Ekg: Cardioscleros levis.

Das Ausscheidungsdiagramm bei der Beobachtung im Mai 1938 ist in Abb. 86 wiedergegeben.

Das Diagramm zeigt am Hungertage deutlich hervortretende endogene  $\beta$ -Oxybuttersäurewellen und verschwindende Zuckerdiurese. Im Gegensatz zur letzteren nimmt die  $\beta$ -Oxybuttersäureausscheidung

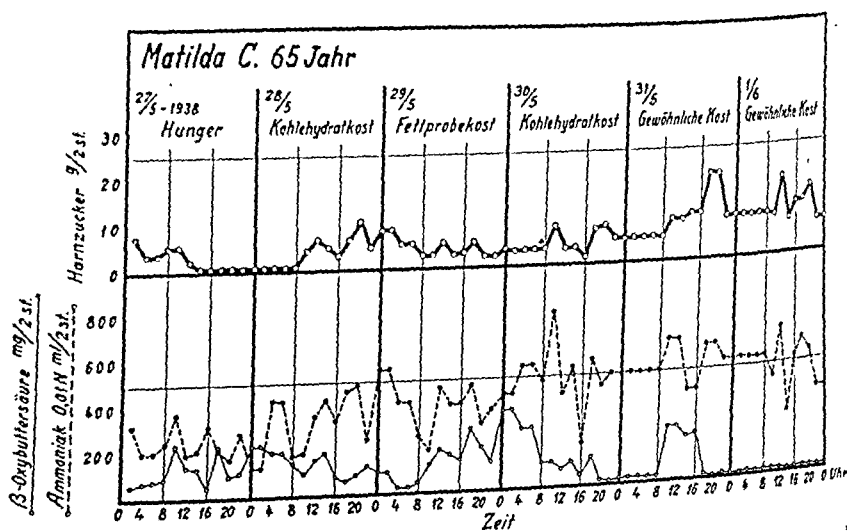


Abb. 86. Ausscheidungsdiagramm bei Diabetes vom Typus A. Im Hunger und bei beschränkter Nahrungszufuhr tritt die Ketonurie hervor, wenn die Zuckerdiurese abnimmt. Bei Kostzulage wird die Zuckerausscheidung stärker, aber die Ketonurie verschwindet.

bei Kohlehydratkost ab und bei Fettkost zu; sie verschwindet am zweiten Tage mit gewöhnlicher gemischer Kost. Die Ammoniakausscheidung weist dabei eine Steigerung auf.

Bei den leichtesten Graden dieses Diabetestyps kann eine Kompensationshyperglykämie dazu genügen, die Ketonkörperbildung in Schach zu halten. Nicht einmal im Hunger macht sich da eine nennenswerte Ketonurie bemerkbar. Sinkt dagegen der Blutzuckerwert, dann beginnt auch hier die eigentliche Stoffwechselstörung hervorzutreten, sie zeigt sich da in Form einer vorübergehenden Ketonurie. Diese verschwindet, wenn der Blutzuckerspiegel bei Kohlehydratzufuhr wieder steigt. Das folgende Beispiel veranschaulicht diesen Typ:

Fall 24. Jan L., geb. 1918.

Keine erbliche Veranlagung für Diabetes. Seit Frühjahr 1935 zeitweise Durstgefühl und Perioden von Müdigkeit. Im Maj 1936 wurde der Diabetes zufällig entdeckt (ärztliche Untersuchung zwecks Bewilligung des Führerscheins für Kraftwagen).

Die hier folgende Abbildung 87 zeigt die Ausscheidungskurven an den Probetagen sowie die Wirkung von Insulin auf die Zuckerdiurese.

Am Hungertage, d. 9. VIII., keine Glykosurie. Der Blutzuckergehalt steigt während des Hungertages auf seinen höchsten Wert, 210 mg %

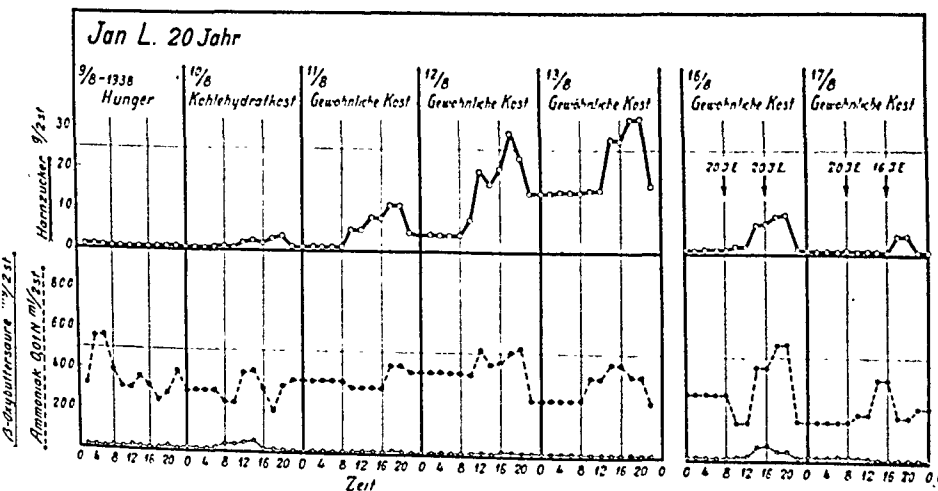


Abb. 87. Ausscheidungsdiagramm bei Diabetes vom Typus A mit sehr schwacher Ketonkörperausscheidung, aber hochgradiger Störung der alimentären Blutzuckerregulation mit starker Zuckerdiurese. Man beachte den günstigen Einfluss des Insulins auf die Zuckerausscheidung (vgl. Abb. 90, S. 338).

um 12 Uhr, und sinkt dann auf 80 mg % um 20 Uhr. Da setzt eine leichte Ketonurie ein, welche im Laufe des folgenden Tages (10. VIII.) bei gesteigerter Kohlehydratzufuhr aufhört. Bei gewöhnlicher Kost stieg die Zuckerdiuresis auf hohe Werte, ohne entsprechende Ketonkörperausscheidung. Als am 15. VIII. Insulin eingesetzt wurde, verschwand die Zuckerausscheidung binnen einiger Tage, nach wie vor bei gewöhnlicher, freier gemischter Kost.

Bei diesen Diabetesformen ist die Zuckerausscheidung sekundär zu einer kompensatorischen Hyperglykämie. Die primäre Schädigung ist bei diesen Fällen eine Störung im Intermediärstoffwechsel infolge von Insulinmangel. Die basale Blutzuckerregulation kann dabei mehr oder weniger schwer gestört sein. Die Hyperglykämie ist bei diesen Fällen als das Äquivalent einer Ketonkörperbildung zu betrachten. In schwereren Fällen reicht der basale Regulationsmechanismus nicht aus, um die Stoffwechselstörung zu kompensieren, wobei die Ketosäurenbildung überhandnimmt und sich bei mangelnder Kohlehydratzufuhr ein Praekoma entwickelt. Der folgende Fall ist ein Beispiel für einen schwereren A-Diabetes mit Praekoma im Anschluss an einen Hungertag, bei dem die Insulinbehandlung wegen der Art der Stoffwechselstörung unentbehrlich war.

*Fall 25. Margit S., geb. 1912.*

Mutter der Pat. leidet an Diabetes. Im Frühjahr 1939 begann die Pat. unter Durstgefühl, Müdigkeit zu leiden und nahm in fünf Monaten 8 kg ab. Im Juli d. J. wurde der Diabetes festgestellt. Diätvorschrift: 75 g Brot, 2 Kartoffeln, 2 Eier, 200 g Sahne, 100 g Fleisch, Butter frei. War bei dieser Diät zeitweise zuckerfrei, aber nach wie vor sehr müde. Konnte jedoch arbeiten. Bei näherer Analyse des Diabetes wurde das hier wiedergegebene Ausscheidungsdiagramm (Abb. 88) erhalten.

Aus dem Diagramm wird ersichtlich, dass eine starke Ketonkörperbildung vorlag, welche am Hungertage eine intensive  $\beta$ -Oxybuttersäureausscheidung ohne entsprechende Ammoniakbildung verursachte. Der pH-Wert des Urins sank während des Hungertages von 5,8 auf 5,0. Gleichzeitig mit der starken  $\beta$ -Oxybuttersäureausscheidung wurde der Urin zuckerfrei, und der Blutzuckerwert sank am Hungertages von 160 auf 65. In der Nacht nach dem Hungertage setzte Übelkeit und praekomatöses Erbrechen ein, am nächsten Morgen Herzbeschwerden, weshalb nach dem Hungertage Insulin gegeben werden musste und eine dauernde Insulinbehandlung eingeleitet wurde.

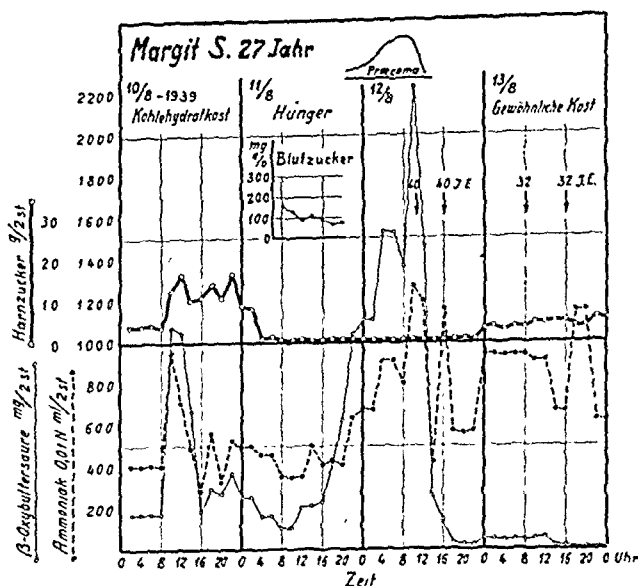


Abb. 88. Ausscheidungsdiagramm bei Diabetes vom Typus A mit sehr intensiver Ketonkörperbildung, so dass sich im Anschluss an einen Hungertag ein praekomatöser Zustand mit Erbrechen und Herzbeschwerden entwickelte. Die Insulinbehandlung musste unverzüglich eingeleitet werden (Indicatio vitalis!).

Bei Fällen von schwererem A-Diabetes mit hochgradigeren Störungen in der basalen Blutzuckerregulation ist der Blutzuckerwert in bezug auf den Zustand irreführend. Bei diesen Fällen kann bei mangelnder Kohlehydratzufuhr der Blutzuckerspiegel auf normale oder subnormale Werte sinken, und zwar gleichzeitig mit einer zunehmenden Ketonkörperbildung. Bei verhältnismässig reichlicher Kohlehydratzufuhr kann in diesen Fällen eine kompensatorische Hyperglykämie der Ketonkörperbildung bis zu einem gewissen Grade entgegenwirken und dadurch den Zustand verbessern. In der hier folgenden Tabelle 30 findet man eine Zusammenstellung von 16 Diabetesfällen vom Typus A mit steigendem Blutzuckerspiegel im Hunger ohne Insulin sowie die entsprechende Zucker- und  $\beta$ -Oxybuttersäureausscheidung teils im Hunger und teils bei gemischter freier Kost ohne Insulin.



## Diabetes vom Typus B

Bei diesem Diabetestyp handelt es sich um eine Störung in der alimentären Blutzuckerregulation. Demgegenüber tritt die primäre Störung im Zwischenstoffwechsel in den Hintergrund. Im Hunger und bei knapper Nahrungszufuhr kann daher der Stoffwechsel in typischen Fällen ganz normal verlaufen. Bei reichlicher Nahrungszufuhr kommt dagegen die Störung der Blutzuckerregulation zum Vorschein. Ketonkörperbildung fehlt sowohl bei Nahrungszufuhr als auch bei kurzdauerndem Hungern.

Ein Diabetes vom Typus B wird dadurch charakterisiert, dass die Zuckerausscheidung im Hunger und bei beschränkter Nahrungszufuhr stark abnimmt oder verschwindet, und dass dabei keine Ketonurie auftritt. Der Blutzuckerwert im Hunger kann normal, subnormal oder leicht erhöht sein, unterliegt aber auch im Hunger grösseren oder geringeren Fluktuationen. Bei Nahrungszufuhr steigt der Blutzuckerwert mehr oder weniger stark, wobei auch eine Glykosurie von wechselnder Intensität auftritt, die sich teilweise mit der zugeführten Kohlenhydratmenge ändert.

Bei den typischen Fällen kommt Ketonurie nicht vor. Im Hunger tritt in diesen Fällen die Stoffwechselstörung zurück. Bei Nahrungszufuhr dagegen tritt die Störung der Blutzuckerregulation mit einer der Kohlenhydratzufuhr entsprechenden Zuckerdiurese in den Vordergrund. Ein Diabetes von Typus B kann daher als ein *Regulationsdiabetes* klassifiziert werden.

Der folgende Fall stellt ein Beispiel für einen B-Diabetes dar:

Fall 26. Nils H., geb. 1927.

Keine Erbanlage für Diabetes. Ein jüngerer Bruder Struma. 1931 Masern und Lungenentzündung, sonst stets gesund gewesen. Im Dezember 1910 zunehmender Durst und Polyurie. Im Laufe des Frühjahrs Müdigkeit und gerigfügige Abmagerung. Im April 1911 wurde der Diabetes festgestellt. 4 Wochen Krankenhausbehandlung. Mit Diät (160 g Brot, 100 g Kartoffeln, 20 g Haferflocken,  $\frac{1}{2}$  l Milch, 75 g Fleisch od. 100 g Fisch, 35 g Butter) sowie Insulin (Leo, Retard) 12 I.E. morgens entlassen. Das Insulin wurde nach 3 Wochen ausgesetzt. Ende Mai 1911 musste wegen steigender Zuckerausscheidung die Insulinbehandlung wiedereingeleitet werden, und zwar mit grös-



serer Dosis (28 I. E. Retard). Am Vormittag Insulinbeschwerden mit Schweissausbrüchen, sonst ziemlich munter. Keine Augen- oder Herzbeschwerden.

4. XII. 1942 Länge 156 cm, Gewicht 47,3 kg. Pulsfrequenz 80, Blutdruck 130/70. Mitralklappenfehler, Inner Organe sonst o.B. Grundumsatz am 5. XII.—29 %.

Der Diabetestypus wird aus dem Ausscheidungsdiagramm ersichtlich (Abb. 89).

Aus der Abbildung geht hervor, dass die Zuckerdiurese am Hungertage (d. 5. XII.) verschwand, ohne dass sich eine Ketonurie oder gesteigerte Ammoniakausscheidung bemerkbar machte. Der Blutzuckergehalt (Bestimmung nach Hagedorn-Jensen) verhielt sich folgendermassen:

	Hunger	Kohlhydratkost	gewöhnl. Kost ohne Insulin
	5. XII.	7. XII.	10. XII.
9 Uhr	226 mg%	— mg%	312 mg%
12 »	184 »	299 »	471 »
14 »	155 »	341 »	529 »
16 »	136 »	287 »	447 »
18 »	96 »	362 »	559 »

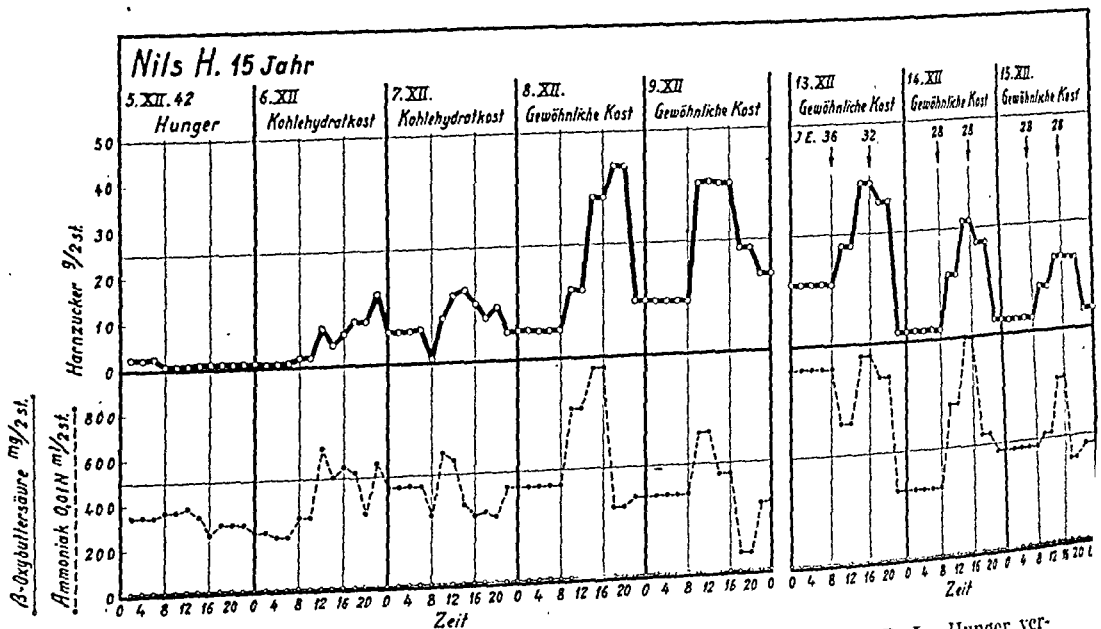


Abb. 89. Ausscheidungsdiagramm bei Diabetes vom Typus B. Im Hunger verschwindet die Zuckerdiurese, ohne dass eine Ketonurie eintritt. Bei Nahrungszufuhr steigt die Zuckerausscheidung mit der zugeführten Kohlehydratmenge. Insulin drückt die Zuckerausscheidung in gewissen Fällen herab, hat aber eine weniger auffällige Wirkung.

Gewicht	5. XII.	.....	46,0 kg	Hunger
	6. »	.....	45,6 »	Kohlhydratprobekost
	7. »	.....	45,4 »	»
	8. »	.....	44,9 »	gewöhnl. Kost.
	9. »	.....	45,1 »	» »
	10. »	.....	44,4 »	» »

Der während des Hungertages erhöhten, auf eine Kompensations-hyperglykämie hindeutenden Blutzuckerwerte und des sinkenden Körpergewichts wegen lag eine relative Insulinindikation vor. Deshalb wurde am 11. XII. trotz fehlender Ketonurie von neuem die Insulinbehandlung eingeleitet. Ausscheidungsdiagramm und Insulinmengen findet man in Abb. 89.

Gleichzeitig mit dem langsamen Nachlassen der Zuckerausscheidung stieg das Gewicht von 44,4 auf 46,7 kg (16. XII.). Man beachte die vorübergehende Zunahme der Ammoniakausscheidung am 12. XII. einen Tag nach der Einleitung der Insulinbehandlung.

Bei gewissen Fällen von B-Diabetes handelt es sich wie hier um eine ungenügende Insulinwirkung im Organismus. Eine Insulinbehandlung ist da mehr oder minder wirksam. Bei anderen Fällen wiederum liegt eine Regulationsstörung vor, welche keinen Insulinmangel verursacht, sondern auf einer abnormen Reizung des blutzuckersteigernden Regulationssystems beruht. Der folgende Fall ist ein Beispiel für einen derartigen B-Diabetes.

*Fall 27. Pehr S., geb. 1920.*

Keine Erbanlage für Diabetes. Während der Wachstumsjahre zeitweise Magenbeschwerden, Diarrhoen, Erbrechen und Neigung zu Azidose, Wuchs in den Jahren 1931—35 rasch und war im Alter von 15 Jahren 177 cm lang.

Erkrankte im Anschluss an eine leichte Parotitis im August 1937 an Diarrhoe und Magenbeschwerden. Nach einigen Tagen zunehmendes Durstgefühl, Müdigkeit und Abmagerung, wobei Diabetes konstatiert wurde. Pat. erhielt zunächst Insulin 2mal tägl. bis 72 I.E. pro Tag, aber trotz dessen stieg das Gewicht nicht, und die Zuckerausscheidung zeigte keine Neigung zum Sinken. Ebenso wenig liess der Zustand im übrigen eine Besserung erkennen, weshalb die Insulindosis herabgesetzt wurde.

Eine nähere Analyse dieses Diabetesfalls mit Ausscheidungsdiagramm während der Zeit 15. V.—9 VI. 1938 ergibt folgendes (s. Abb. 90): Während der Probetage mit Nahrungsentziehung und reduzierter Kost fehlte jegliche Zuckerausscheidung. Eine geringfügige vorüber-

gehende Ketonkörperausscheidung trat am Tage nach dem Hungertag (16. V.) auf. Am Hungertage war der Blutzuckergehalt niedrig, er variierte zwischen 110 und 40 mg %. Bei gewöhnlicher gemischter, freier Kost stellte sich eine mässige Zuckerdiurese ein, und der Blutzuckergehalt schwankte zwischen 140 und 290 mg %.

Nach dem Hungertag ohne Insulin am 16. V. betrug das Körpergewicht 64,1 kg, stieg aber während der folgenden insulinfreien Tage bis auf 61,5 kg am 23. V. Am nächsten Tage wurde die Insulinbehandlung eingeleitet. Trotz freier Nahrungszufuhr und Insulin 1- bis 2mal tägl., mit Dosen bis 48 I.E. pro Tag, stieg das Körpergewicht nicht, sondern begann statt dessen zu sinken und betrug am 9. VI. 1938 61,9 kg. Auch die Zuckerausscheidung nahm merkbar zu. Am 30. V. variierte der Blutzuckergehalt zwischen 220 und 400 mg %, trotz zweier Insulindosen von je 20 I.E. um 8 bzw. 16 Uhr. Das Insulin bewirkte auch sonst keinerlei Besserung der übrigen Diabetes-symptom des Kranken oder des subjektiven Allgemeinzustandes desselben.

Dieser Fall wurde dagegen von Vitamin B günstig beeinflusst.

Werden bei diesen Fällen Kohlehydrate zugeführt, welche als Glykose resorbiert werden, so kann der Zuckergehalt des Blutes auf hohe Werte steigen. Dabei tritt eine mehr oder we-

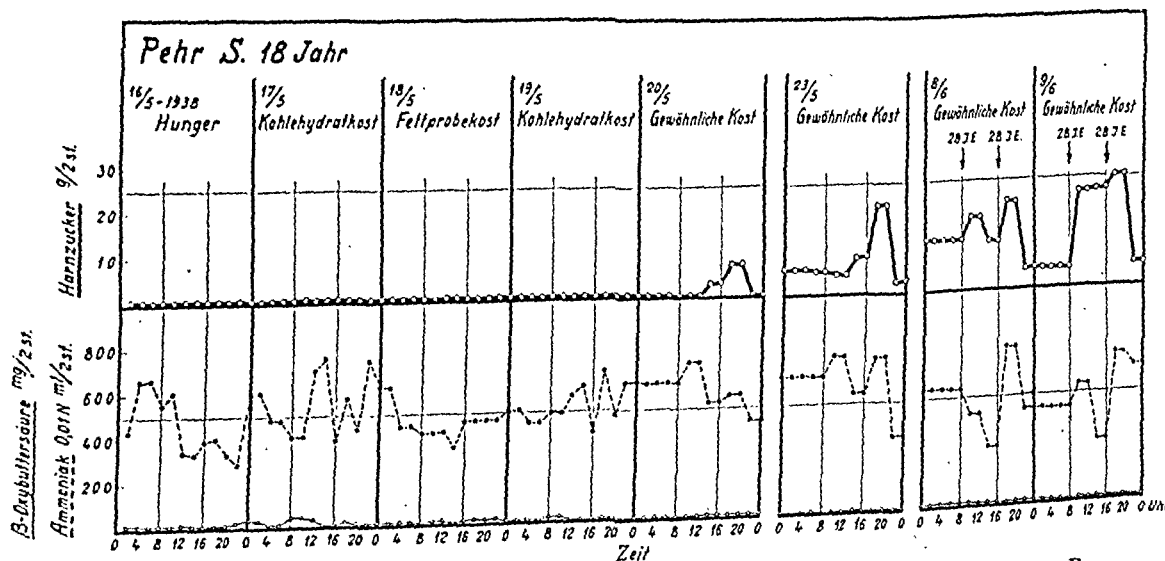


Abb. 90. Ausscheidungsdiagramm bei insulinresistentem Diabetes vom Typus B. Abgesehen von einer sehr unerheblichen, vorübergehenden Ketonurie im Anschluss an den Hungertag keine Ausscheidung von Ketonkörpern. Im Hunger und bei beschränkter Nahrungszufuhr verschwindet die Zuckerdiurese, kommt aber bei Kostzulage wieder. Insulin steigert bei diesem Falle die Zuckerausscheidung. Ammoniakausscheidung unverändert.

niger starke Glykosurie auf, je nach der Art des Diabetesfalles und der zugeführten Kohlehydratmenge. Da Fett bei der Verdauung nicht direkt in Glykose übergeht, ist die Blutzuckersteigerung bei Fettresorption geringer als bei kohlehydratreicher Kost. Fett bewirkt infolgedessen eine weniger starke Glykosurie als Kohlehydrate und wird daher als Nahrung besser ausgenützt, sofern der Fettstoffwechsel normal ist und Nierenkörperausscheidung fehlt.

Die folgende Tabelle 31 enthält eine Zusammenstellung von 16 Diabetesfällen vom Typus B, nach steigendem Blut-

TAB. 31.

Blutzuckergehalt, Harnzucker und  $\beta$ -Oxybuttersäureausscheidung bei 16 Fällen von B-Diabetes ohne Insulin an einem Hungertage und an einem Tage mit gemischter freier Kost.

	H u n g e r						F r e i e K o s t					
	Blutzucker, Mittel aus höchstem und niedrigstem Wert während des Tages	Ausscheidung im Urin 8—20 Uhr				Blutzucker, Mittel aus höchstem und niedrigstem Wert während des Tages	Ausscheidung im Urin 8—20 Uhr					
		Zuk- ker	$\beta$ -Oxy- butter- säure	H <sub>2</sub> N	Ge- samt- azidi- tät		Zuk- ker	$\beta$ -Oxy- butter- säure	H <sub>2</sub> N	Ge- samt- azidi- tät		
	mg %	g	mg	ml $\frac{n}{10}$	ml $\frac{n}{10}$	mg %	g	mg	ml $\frac{n}{10}$	ml $\frac{n}{10}$		
Einar J. ....	65 $\pm$ 25	0	0	128	129	93 $\pm$ 37	11,8	0	305	278		
Gunnar K. ....	66 $\pm$ 28	2,1	0	159	125	276 $\pm$ 121	81,4	0	258	215		
Einar E. ....	87 $\pm$ 24	0	0	197	202	190 $\pm$ 35	4,6	0	254	235		
Lotten F. ....	98 $\pm$ 55	0,2	0	91	130	325 $\pm$ 27	31,3	0	215	161		
Sigrid E. ....	100 $\pm$ 24	0	0	109	142	247 $\pm$ 52	5,1	0	146	187		
Oskar H. ....	105 $\pm$ 25	0	0	57	74	197 $\pm$ 38	4,7	0	22	9		
Elisabeth L. ....	110 $\pm$ 50	0	0	150	176	165 $\pm$ 75	3,1	0	250	283		
Gunnar L. ....	116 $\pm$ 20	0	0	169	117	169 $\pm$ 19	5,0	0	240	152		
Sune K. ....	120 $\pm$ 40	0	0	165	112	175 $\pm$ 45	98,6	0	302	81		
Fritjof L. ....	121 $\pm$ 20	0	0	229	192	185 $\pm$ 34	2,2	0	256	161		
Sture N. ....	130 $\pm$ 40	0	0	137	77	233 $\pm$ 127	24,6	0	163	142		
Hans S. ....	130 $\pm$ 25	0,3	0	121	142	220 $\pm$ 60	31,5	0	216	285		
Carl U. ....	145 $\pm$ 32	0	0	218	232	316 $\pm$ 69	95,6	0	216	244		
Bror S. ....	166 $\pm$ 39	3,1	0	188	283	360 $\pm$ 58	50,8	0	261	291		
Anna-Lisa L. ....	197 $\pm$ 65	0	0	126	111	341 $\pm$ 106	9,6	0	171	131		
Hanna H. ....	246 $\pm$ 50	0,2	0	103	99	372 $\pm$ 26	16,3	0	120	105		

zuckerspiegel im Hunger ohne Insulin geordnet, mit entsprechenden Fluktuationen des Blutzuckerwerts und der Zuckerausscheidung bei gemischter freier Kost ohne Insulin. Der Blutzucker ist als Mittel des höchsten und niedrigsten Wertes von fünf Blutzuckerbestimmungen nach Hagedorn um 9 bzw. 12, 14, 16 und 18 Uhr angegeben.

Durch die Glykosurie verliert der Organismus Kohlehydrate. Hierdurch kann ein Kohlehydratmangel im Körper eintreten. Wird dieser zu gross, so fehlen Kohlehydrate zur Glykogenbildung, wobei Fett Material für diese liefern muss. Wenn die Kohlehydratverbrennung dabei unzulänglich ist, kommt es zu Störungen in der normalen Umsetzung der Fettsäuren, wodurch die Kohlehydrateinbusse des Organismus zum Anlass einer Ketonkörperbildung werden kann, ohne dass irgendeine primäre Störung im Zwischenstoffwechsel vorzuliegen braucht. Bei reichlichem Zugang zu Kohlehydraten in der Nahrung kann in diesen Fällen die Ketonkörperbildung vermieden werden, aber bei kohlehydratarmer Kost kann ein Diabetes von diesem Typus in einen ketonkörperbildenden Diabetes von mehr oder weniger bedrohlichem Charakter übergehen. Beruht eine Ketonkörperbildung auf einer ungenügenden Kohlehydratzufuhr in der Kost, so verschwindet dieselbe bei Kohlehydratzulage. Der folgende Fall ist ein Beispiel für dieses Verhalten:

*Fall 28. Sven A., geb. 1878.*

Keine Erbanlage für Diabetes. 1924 wurde der Diabetes konstatiert, es wurde strenge Diät verordnet, vorwiegend Fett-Gemüse, nur wenig Brot, keine Kartoffeln. Pat. hielt diese Diät 10 Jahre lang, war zeitweise müde und litt an rezidivierenden Iritiden, war aber im grossen ganzen ziemlich munter und arbeitete.

Bei der Beobachtung im März 1934 lag ein Diabetes mit erheblicher  $\beta$ -Oxybuttersäureausscheidung vor. Diese nahm im Laufe einiger Kohlehydratprobekosttage ab (Abb. 91). Pat. erhielt nun eine kohlehydratreiche, freiere Kost, und sein Zustand besserte sich merkbar, er war weniger müde. Auch die Augenbeschwerden verschwanden. Kein Durstgefühl. Bei neuerlicher Beobachtung im September 1937 war jegliche  $\beta$ -Oxybuttersäureausscheidung verschwunden; diese trat aber vorübergehend im Anschluss an Fettbelastung nach einem Fettprobekosttage mit reduzierter Kohlehydratmenge wieder hervor (Abb. 91, unteres Diagramm, 17.—18. IX.).

Bei einer gewöhnlichen freien gemischten Kost, mit etwas eingeschränkter Fettmenge, lag nur eine mässige Zuckerdiaurese vor, aber keine Ketonurie.

Ist durch hochgradigen Kohlehydratmangel im Organismus oder durch irgendeine andere Ursache die Glykogenbildung gestört worden, mit dauernder Schädigung des Leberparenchyms, dann lässt sich die Ketonkörperbildung nicht durch

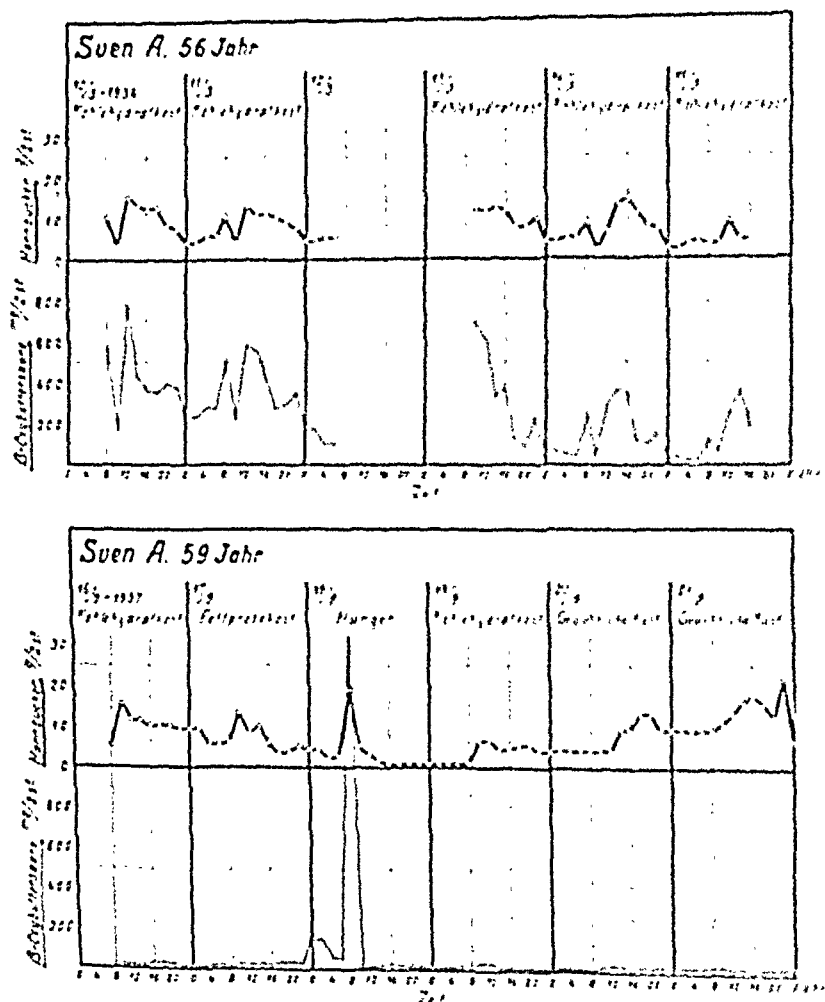


Abb. 91. Ausscheidungsdiagramme bei Diabetes mit Kohlehydratmangelernährung. Abnahme der Ketonurie bei kohlehydratreicherer Kost. Nach dreijähriger freier und kohlehydratreicherer Kost war die Ketonurie fast verschwunden, kam aber temporär im Anschluss an einen Fettbelastungstag wieder,

vermehrte Kohlehydratzufuhr unterdrücken. Ein Diabetes vom Typus B kann dabei in einen bedrohlichen Diabetes übergehen.

Solange die Glykogenbildung der Leber bei einem Regulationsdiabetes verhältnismässig ungestört ist, spiegelt sich die wechselnde Kohlehydrataffinität dieses Organs in Variationen des Blutzuckergehalts und auch in den Ausscheidungsdiagrammen mehr oder minder deutlich wider. Die rhythmische Lebertätigkeit erscheint dabei am deutlichsten, wenn die zugeführte Kohlehydratmenge der Assimilationskapazität der Leber entspricht und die Toleranzgrenze daher nur wenig überschreitet. Wird die Kohlehydratzufuhr zu gross, so kommt die periodische Leberfunktion in der Blutzuckerkurve und im Zuckerausscheidungsdiagramm weniger deutlich zum Ausdruck (vgl. Abb. 17 S. 67).

Über die Wirkung des Insulins bei Diabetesformen vom Typus B vgl. IX. Abschnitt S. 384.

## Diabetes vom Typus O

Ist ein Patient mit Insulin behandelt und die Dosis Schritt für Schritt herabgesetzt oder an einem Hungertage weggelassen worden, dann gibt es vier Möglichkeiten: Bei einer Reihe von Fällen tritt beim Aussetzen des Insulins ein Diabetes vom Typus A oder B in Erscheinung. Bei anderen Fällen kann das Insulin auch weiterhin entbehrlich sein, ohne dass sich irgendwelche Anzeichen eines Diabetes bemerkbar machen. Vorausgesetzt, dass es sich wirklich um einen Diabetes gehandelt hatte, als die Insulinbehandlung eingeleitet wurde, und dass das Insulin nicht aus anderen Gründen verabreicht worden war, hat bei diesen Fällen ein transitorischer Diabetes vorgelegen, der mit der Zeit verschwunden ist, wenngleich die Insulinbehandlung weiterging. In noch anderen Fällen tritt bei Herabsetzung der Insulindosis plötzlich eine starke Ketonkörperbildung ein, ohne entsprechende Ammoniakbildung, und infolgedessen entwickelt sich rasch ein Praekoma, wenn die Insulinbehandlung nicht wieder effektiv gemacht wird. Diese Diabetesfälle können als *Diabetes vom Typus O* (keine (0) insu-

linfreien Tage) oder *bedrohlicher Diabetes* bezeichnet werden. Wenn bei diesen Fällen das Insulin ausgesetzt wird, sinkt infolge der ungenügenden Ammoniakbildung der pH-Wert des Urins, wobei das Nierenepithel geschädigt werden kann. Dies zeigt sich im Auftreten einer Albuminurie mit Zylindern im Harn (S. 248). Durch Entwicklung von Ketosäuren verschlimmert sich bei mangelnder Insulinwirkung der Zustand rasch. Hier liegt eine schwere Störung sowohl in den regulatorischen Systemen wie im Zwischenstoffwechsel vor. Was primär ist, was sekundär, das zu entscheiden ist nicht möglich. *Die intensive Ketonkörperbildung und die ungenügende Ammoniakbildung sind für einen bedrohlichen Diabetes vom Typus 0 charakteristisch.*

Bei sinkendem Blutzuckerspiegel im Hunger wird die Ketonkörperbildung sehr rasch gesteigert. Im Gegensatz zum A-Diabetes vermag Kohlehydratzufuhr die Ketonkörperbildung nicht herabzudrücken. Dies ist nur durch Insulingaben möglich. Abb. 51 S. 267 zeigt einen Übergang vom Typus A zum bedrohlichen Diabetes, mit bei Nahrungszufuhr noch mehr gesteigerter Ketosäurenbildung, aber mit andauernd hinreichender Ammoniakproduktion.

Wenn die Ammoniakbildung nicht mit der Zunahme der Ketonkörper Schritt halten kann, sondern der Ammoniak- $\beta$ -Oxybuttersäurequotient unter 1 sinkt, dann entwickelt sich in kurzer Zeit ein Praekoma (S. 246). Der folgende Fall 29 ist ein Beispiel für einen Diabetes von diesem Typ:

Fall 29. Bertil B., geb. 1895.

Keine Erbanlage für Diabetes. Früher gesund gewesen.

Oktober 1932 lästiger Harndrang, nach einer Woche starker Durst (trank täglich 7–8 l), Schwächegefühl und beginnende Abmagerung (10 kg in 1 Monat). Diabetes konstatiert, zwei Wochen Krankenhausbehandlung. Mit strenger Diät (50 g Brot) und Insulin 12 + 12 I.E. entlassen. Zunehmende Abmagerung und Schwäche. Im Herbst 1935 reichlichere Kost, Insulin durch Synthalin ersetzt. Wegen Magenbeschwerden hörte Pat. selbst auf, Synthalin einzunehmen, und begann wieder mit Insulin, 16 + 16 I.E. Zeitweise Durstgefühl und seit Sommer 1938 Brennen und Empfindlichkeit der Haut an Füßen und Unterschenkeln. Bei der Beobachtung im November 1938 Länge 169 cm, Gewicht 66,2 kg. Blutdruck 120/75, Puls-



Frequenz 76. Herz: Grenzen  $2\frac{1}{2}$  u.  $10\frac{1}{2}$  cm, Töne dumpf, 1. Ton über der Herzspitze gespalten. Keine Arrhythmie. Ekg: QRS verbreitert, arborisiert, ST gesenkt,  $T_a$  neg. Überleitungszeit 0,16 Sek. Nervensystem: Hyperästhesie an Füßen und Unterschenkeln. Bauchreflexe auf der linken Seite abgeschwächt, Reflexe sonst o. B. Der Diabetestypus wird aus dem Ausscheidungsdiagramm (Abb. 92) ersichtlich.

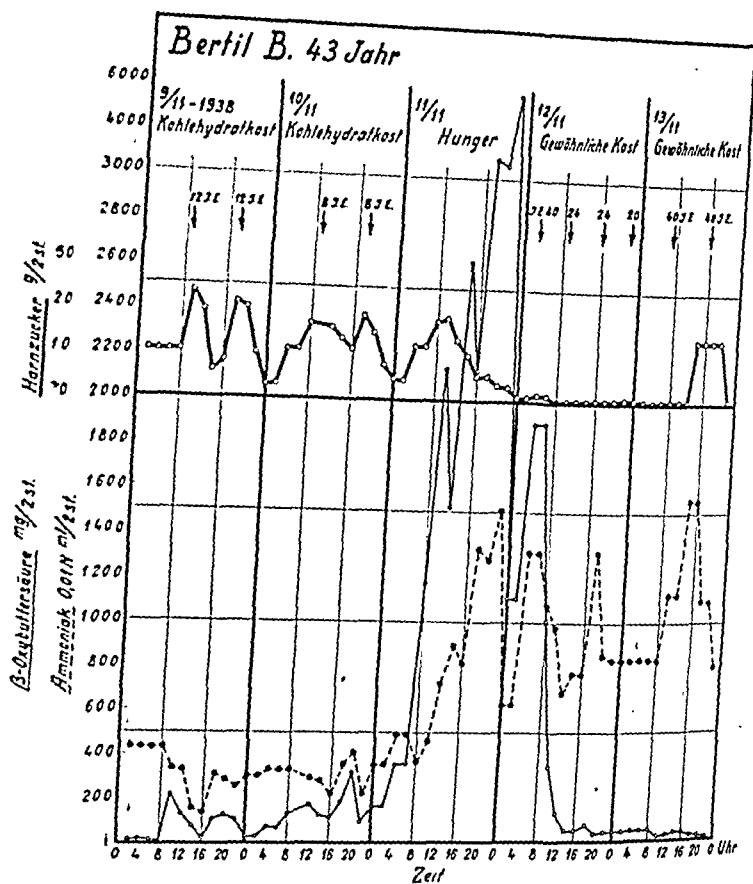


Abb. 92. Ausscheidungsdiagramm bei Diabetes vom Typus O. Während des Hungertages ohne Insulin starke  $\beta$ -Oxybuttersäureausscheidung mit mangelhafter Ammoniakbildung. In der Nacht nach dem Hungertage setzten präkomatöse Beschwerden (Erbrechen, Herzklopfen und Tachykardie) ein, im Anschluss an die endogene Ketosäurenwelle, die sich nach 2 Uhr an dem auf den Hungertag folgenden Tage zu entwickeln begann. Am 12. XI. wurde um 3.15 Uhr die Insulinbehandlung eingeleitet. Die Ketonurie wurde rasch geringer, hörte aber während der nächsten 24 Stunden nicht ganz auf, dagegen verschwand bei der forcierten Insulinbehandlung die Zuckerausscheidung am Tage nach dem Hungertage. Die präkomatösen Beschwerden waren nach einigen Stunden völlig verschwunden, und der Kranke war bereits am Tage nach dem Hungertage beschwerdefrei.

Veränderungen des Blutzuckerwerts, der Harnzucker-,  $\beta$ -Oxybutter-säure- und Ammoniakausscheidung sowie des pH-Werts im Urin bei 16 Fällen von bedrohlichem Diabetes (Typus 0) während eines Hungertages mit beginnendem Prækoma.

	Alter	Blutzuckerwert mg %								Ausscheidung im Urin 8—20 Uhr				pH im Urin		Prækoma	Insulin Uhr
		Uhr								Zucker g	$\beta$ -Oxy- butter- säure mg	$\text{H}_2\text{N}$ - Milli- mol	Ge- samt- azidität Milli- mol	um 8 Uhr	bei Beginn des Prækomas		
		8	9	10	12	14	16	18									
Lars J. ....	11	—	258	—	233	214	209	181	9,9	5121	34,6	39,3	5,9	5,4	19	Übelkeit, Herzklopfen, Erbrechen	22,00
Tora E. ....	17	170	—	240	220	240	250	190	8,6	8012	65,8	59,6	5,7	5,3	18	Übelkeit, Erbrechen	19,00
Erik S. ....	37	—	351	—	298	305	291	218	40,8	4038	30,1	36,1	6,4	5,5	16	Herzbeschwerden mit Prækordialangst	18,00
Axel R. ....	46	210	—	220	220	240	285	220	62,2	10029	51,0	75,4	5,6	5,4	16	Kopfschmerzen, Übel- keit, Erbrechen	19,00
David P. . .	37	190	—	210	205	180	180	240	46,7	5628	38,0	63,6	5,2	5,1	18	Übelkeit, Erbrechen	19,30
Rune H. ....	13	240	—	240	238	240	—	—	49,6	4106	34,4	41,6	5,7	5,3	10	Kopfschmerzen, Übel- keit, Erbrechen	15,00
Ingela E. . .	9	200	—	280	260	210	240	—	2,5	3570	47,0	41,0	5,7	5,5	15	Übelkeit, Erbrechen	16,00
Karl P. ....	17	—	374	—	334	247	240	291	42,6	8586	45,6	60,7	5,4	5,3	17	Brechreiz, Herzklopfen	19,45
Astrid B. . .	30	230	—	220	280	230	295	—	18,9	7881	39,1	52,6	5,9	5,6	16	Übelkeit, Erbrechen	17,30
Herta, A. . .	38	360	—	230	305	355	310	295	84,6	10304	32,9	50,1	5,8	5,5	17	Übelkeit	20,30
Erik K. ....	32	250	—	250	285	285	300	—	73,7	10669	60,4	102,9	5,6	5,4	12	Übelkeit, Erbrechen	17,30
Mauritz G. .	31	—	350	—	393	292	327	—	23,3	9604	56,9	73,5	5,7	5,1	14	Übelkeit, Herzklopfen, Erbrechen	16,15
Eivor F. ....	23	320	—	370	310	330	—	—	60,6	9828	65,6	85,1	5,1	4,9	12	Übelkeit, Herzklopfen, Erbrechen	13,30
Bror N. ....	36	—	439	—	404	392	378	374	58,6	7393	32,2	58,4	5,7	5,4	19	Übelkeit, Erbrechen	19,30
Margareta D	18	—	247	—	405	395	—	—	41,8	14368	59,2	63,4	5,6	5,3	11	Übelkeit, Erbrechen	14,00
Herman H. .	33	—	479	—	461	—	—	—	58,8	9502	64,4	67,2	5,6	5,2	10	Übelkeit, Herzklopfen	12,10

Der Blutzuckergehalt nach Hagedorn war am Hungertage (11. XI.) ohne Insulin sowie am 15. XI. bei freier gemischter Kost und 40 + 32 I. E. Insulin, den endogenen Ketosäurenwellen angepasst:

Blutzuckergehalt	11. XI. Hunger	15. XI. gewöhnl. Kost + Insulin
8 Uhr	260 mg%	170 mg%
10 „	250 „	80 „
12 „	260 „	220 „
14 „	260 „	240 „
16 „	220 „	250 „
18 „	220 „	125 „
20 „	160 „	90 „

Das Gewicht sank nach dem Hungertage auf 61,5 kg, stieg aber nach Einleitung der Insulinbehandlung wieder auf 66,5 kg. nach 9 Tage.

Der Insulinbedarf im weiteren Verlauf betrug bei diesem Fall 28 + 28 I. E. in 24 Stunden.

Der Blutzuckergehalt hat keine direkte Bedeutung für die Entwicklung des Praekomas. Dies wird daraus ersichtlich, dass der Blutzuckerwert vor dem Eintritt des Praekomas sowohl eine fallende wie steigende Tendenz aufweisen kann. Die vorstehende Tab. 32 enthält 16 Fälle von 0-Diabetes, bei welchen die praekomatösen Beschwerden während eines Hungertages mit ungenügender Insulinwirkung auftraten. In der Tabelle sind die Veränderungen des Blutzuckerspiegels vor dem Einsetzen des Insulins sowie die pH-Senkung des Urins vor dem Praekoma angegeben. Die Tabelle macht auch die Ausscheidung von Zucker,  $\beta$ -Oxybuttersäure, Ammoniak und der Gesamtsumme saurer Valenzen in Millimol bei pH 9,0 ersichtlich (Phenolphthalein als Indikator). Insulin wurde nach Beginn des praekomatösen Erbrechens eingesetzt.

### Häufigkeit der verschiedenen Diabetestypen

Bei der Analyse von 1390 Diabetesfällen aus den Jahren 1935—1940 (748 Männer und 642 Frauen) ergab sich folgende Verteilung auf die verschiedenen Diabetestypen (Tab. 33):

TAB. 33.

Verteilung der einzelnen Diabetestypen bei 1390 Fällen von Diabetes.

Diabetestypus	Männer		Frauen	
	Anzahl	%	Anzahl	%
Typus A .....	326	43,6	220	34,4
Typus B .....	191	25,5	171	26,6
Typus O .....	231	37,9	251	39,0
Anzahl Fälle	748	100 %	642	100 %
Sa.	1390 Fälle			

### Altersfrequenz für den Ausbruch der klinischen Symptome bei den verschiedenen Diabetestypen

Das Alter der Patienten beim Auftreten der Diabetessymptome geht aus der hier eingefügten Tabelle 34 für die verschiedenen Diabetestypen hervor (1390 Fälle).

Hieraus wird ersichtlich, dass die Altersfrequenz für Diabetes vom Typus B und O ganz verschieden ist.

Für B-Diabetes finden wir bei Männern ein starkes Maximum im Alter von ungefähr 15—20 sowie 50—55 Jahre. Bei Frauen ist ein angedeutetes Maximum in der Gruppe 15—20 Jahre zu bemerken, aber das höchste Frequenzmaximum fällt zwischen 50 und 60 Jahre.

Für O-Diabetes weist dagegen die Frequenzkurve bei sowohl Männern und Frauen ein starkes Maximum im Alter 5—15 Jahre auf. Dann sinkt die Kurve des O-Diabetes ziemlich gleichmässig mit steigendem Lebensalter.

Schon in der Alterskurve für das Auftreten der verschiedenen Diabetestypen macht sich mithin ein prinzipieller Unterschied geltend, was auf einen verschiedenen Ursprung der beiden Diabetesformen, des Typus O und B, hindeutet.

Das Frequenzmaximum des Typus O fällt mit einer Wachstumsperiode bei beiden Geschlechtern zusammen. Unter der Voraussetzung, dass die *Bomskowsche* Anschauung über das

TAB. 34.  
Alter beim Ausbruch der Krankheit.

Ausgaben der Krankheit.										
Alter	Männer				Frauen				Sa. Männer u. Frauen	
	Anzahl				Anzahl					
Typus	A	B	0	Sa.	A	B	0	Sa.		
0— 5 Jahre....	4	—	26	30	8	—	17	25	55	
5—10 „ ....	14	3	36	53	10	4	43	57	110	
10—15 „ ....	34	9	42	85	18	6	39	63	148	
15—20 „ ....	33	33	25	91	16	9	26	51	142	
20— 25 „ ....	26	11	16	53	20	7	26	53	106	
25—30 „ ....	23	17	21	61	19	2	17	41	102	
30—35 „ ....	33	15	18	66	16	7	22	45	111	
35—40 „ ....	38	15	15	68	14	9	11	34	102	
40—45 „ ....	28	11	11	50	15	12	14	41	91	
45—50 „ ....	35	22	4	61	21	15	13	49	110	
50—55 „ ....	23	24	6	53	21	31	9	61	114	
55—60 „ ....	19	12	7	38	15	30	7	52	90	
60—65 „ ....	11	12	1	24	18	15	4	37	61	
65—70 „ ....	4	5	3	12	6	10	2	18	30	
70—75 „ ....	1	1	—	2	1	8	—	9	11	
75—80 „ ....	—	—	—	—	2	3	—	5	5	
80—85 „ ....	—	1	—	1	—	—	1	1	2	
Sa.	326	191	231	748	220	171	251	642	1390	

Thymushormon als kombiniertes Wachstums- und Diabetes hervorrufendes Hormon zutrifft, kann die Anhäufung der bedrohlichen Diabetesfälle im Alter 5—15 Jahre ihre Erklärung finden.

Der Diabetes vom Typus B bevorzugt dagegen einmal das Alter 15—20 Jahre, sodann das Alter 50—55 Jahre, wobei in der ersten Altersgruppe die Männer, in der zweiten dagegen die Frauen überwiegen. In diese Zeitabschnitte fallen Pubertät, Nachpubertät und Klimakterium. Ein B-Diabetes ist daher hinsichtlich seiner Entstehung wahrscheinlich in irgendeiner Weise mit der Tätigkeit der Geschlechtsdrüsen verknüpft.

Der Diabetes vom Typus A nimmt eine Zwischenstellung zwischen den beiden anderen Typen ein, mit weniger ausgeprägten Höhepunkten zur Zeit der Pubertät und des Klimakteriums.

## Diabetes und Jahreszeiten

Es ist von Interesse, zu sehen, ob eine bestimmte Jahreszeit eine besondere Disposition für Diabetes schafft. Die hier eingeschobene Tab. 35 macht ersichtlich, in welchem Monat die klinischen Diabetessymptome bei 991 Fällen von Diabetes eingesetzt hatten.

TAB. 35.  
Jahreszeit des Beginns klinischer Diabetessymptome.

M o n a t	Anzahl Fälle			
	Diabetestypus			Sa.
	A	B	0	
Januar .....	54	20	26	100
Februar .....	39	17	18	75
März .....	41	11	28	80
April .....	47	15	20	82
Mai .....	29	12	18	59
Juni .....	33	16	31	80
Juli .....	33	17	13	63
August .....	41	20	16	77
September .....	41	18	27	86
Oktober .....	69	14	29	112
November .....	35	16	25	76
Dezember .....	45	21	36	102
Sa. Diabetesfälle	507	287	197	991

Eine ausgeprägte jahreszeitliche Disposition scheint für den Ausbruch des Diabetes nicht vorzuliegen, möglicherweise nehmen die bedrohlichen Fälle im Herbst und Frühjahr etwas zu.

## Die Bedeutung der Heredität für die Entstehung des Diabetes

Die komplizierten Verhältnisse, die beim Diabetes obwalten, die wechselnde und uneinheitliche Genese der Krankheit, machen wahrscheinlich, dass man nicht mit einfachen Verer-

lungsgesetzen für Diabetes rechnen kann. Hierzu kommt noch die verschiedene Altersdisposition für die einzelnen Diabetes-typen, wodurch die Beurteilung der Diabeteserblichkeit in noch höherem Grade erschwert wird. Eine Häufung von Diabetes-fällen in einer Familie kann zufallsbedingt sein und braucht nicht den Ausdruck irgendeines erblichen Faktors darzustellen. Auf der anderen Seite muss man in Betracht ziehen, dass es eine erbliche konstitutionelle Schwäche der glykoregulatorischen Systeme geben kann, welche zum Diabetes disponiert.

Mein eigenes, diese Frage beleuchtendes Diabetesmaterial, stammt aus 1220 Familien mit von 1 bis 16 Kinder. Die Gesamtanzahl der Kinder betrug in diesen Familien 5932. Von diesen Kindern litten 1449 ( $\approx 24,4\%$ ) an Diabetes in irgendeiner Form. Teilt man diesen Prozentsatz mit Rücksicht auf das Vorkommen von Diabetes bei den übrigen Familienmitgliedern in Gruppen ein, so ergibt sich folgendes (Tab. 36):

TAB. 36.  
Bedeutung der Heredität für die Diabetesfrequenz.

Diabetesheredität	Familienanzahl	Kinderanzahl		Diabetes in Prozent der gesamten Kinderanzahl
		insgesamt	davon Diabetiker	
<i>Diabetes in d. Familie nicht bekannt</i> .....	857	4153	986 Fälle	23,7 %
<i>Diabetes in d. Familie:</i>				
Vater Diabetiker .....	89	455	124 "	27,3 %
Mutter " .....	82	538	113 "	21,0 %
Beide Eltern " .....	6	30	14 "	46,7 %
Grosseltern " .....	57	199	68 "	34,2 %
Sonstige Blutsverwandte... (Onkel, Tanten, Vettern od. Basen)	129	557	144 "	25,9 %

Bei der Beurteilung der verhältnismässig niedrigen Diabetesfrequenz in Familien, wo die Mutter an Diabetes leidet, muss man sich die Tatsache vor Augen halten, dass die Säuglingssterblichkeit bei zuckerkranken Müttern höher ist als bei nicht zuckerkranken. Die relativ höhere Diabetesfrequenz in Fami-

lien, in welchen Diabetes sonst vorkommt, spricht für einen gewissen Grad von Erblichkeit bei Diabetes. Zu einer sichereren statistischen Bearbeitung dieser Frage ist jedoch ein grösseres Material erforderlich, welches zugleich mit Rücksicht auf die verschiedene Genese des diabetischen Zustandes vollständig bearbeitet ist. Ein derartiges Material steht zur Zeit noch aus.

### Variationen des Nüchternblutzuckerwerts bei den verschiedenen Diabetestypen

An insulinfreien Hungertagen schwankt der Blutzuckerspiegel unter dem Einfluss einerseits der periodischen Glykogenbildung in der Leber und andererseits der regulatorischen neurohormonalen Periodizität. Alle diese Vorgänge verschmelzen zu dem endogenen Rhythmus des Kohlehydratstoffwechsels (vgl. S. 273). Der Blutzuckernüchternwert ist daher bei Regulationsstörungen keine konstante Grösse, sondern wechselt an verschiedenen Zeitpunkten im Laufe der 24Stundenperiode. Diese Veränderungen des Blutzuckergehalts sind 24stundenrhythmischen Variationen unterworfen (vgl. S. 79).

Um einen Begriff von den Grenzen zu erhalten, innerhalb welcher der Nüchternblutzuckerwert bei verschiedenen Diabetesfällen variiert, wurden 856 Fälle an einem Hungerlage von 8 bis 18 Uhr zweistündlich mit Blutzuckerbestimmungen nach Hagedorn untersucht. Während dieser Zeit wurde keine Nahrung verabreicht, nur Wasser gegen den Durst. Die Kranken lagen zu Bett. Die Verteilung der Diabetesfälle auf die verschiedenen Typen war folgende:

Typus A	.....	432	Fälle
» B	.....	293	»
» O	.....	131	»
<hr/>			
Sa. 856 Fälle			

Die Grenzen, innerhalb welcher der Nüchternblutzuckerwert bei den einzelnen Diabetesfällen schwankte, werden aus den hier eingeschobenen Tabellen 37, 38 und 39 ersichtlich. Bei den bedrohlichen Diabetesfällen vom Typus O war die Insulindosis während der unmittelbar vorangehenden Tage Schritt für



TAB. 37.

Grenzen, innerhalb welcher der Nüchternblutzuckerwert bei 432 Fällen von A-Diabetes an einem Hungertag schwankte.

Niedrigster Blutzucker- wert mg %		Anzahl Fälle	H ö c h s t e r   B l u t z u c k e r w e r t																					
			80	100	120	140	160	180	200	220	240	260	280	300	320	340	360	380	400	420	440	460	480	500
40	10	1	1	4	1	8	2	1	1	2	2	1	1	2	1	1	1	1	1	1	1	1	1	1
60	30		4	7	8	8	12	6	7	2	8	3	2	2	1	2	2	1	1	1	1	1	1	1
80	53		3																					
100	61			3	4	12	14	7	11	5	8	3	2	2	1	2	2	1	1	1	1	1	1	1
120	86				3	9	17	21	20	10	8	3	2	2	1	2	2	1	1	1	1	1	1	1
140	52					2	3	9	10	5	16	5	6	4	2	2	2	1	1	1	1	1	1	1
160	44							1	5	10	5	3	4	2	1	1	1	1	1	1	1	1	1	1
180	26								3	10	5	5	5	2	1	1	1	1	1	1	1	1	1	1
200	27									1	5	3	2	2	4	2	1	1	1	1	1	1	1	1
220	15																							
240	15																							
260	10																							
280	2																							
300	1																							
432		1	8	21	21	39	51	48	57	63	37	24	26	9	8	9	2	3	2			2	1	1

TAB. 38.

Grenzen, innerhalb welcher der Nüchternblutzuckerwert bei 293 Fällen von B-Diabetes an einem Hungertag schwankte.

H ö c h s t e r   B l u t z u c k e r w e r t																								
Niedrigster Blutzucker- wert mg %	Anzahl Fälle	80	100	120	140	160	180	200	220	240	260	280	300	320	340	360	380	400	420	440	460	480	500	
		40	11	2	5	3			1	2	1				3									
60	34	2	4	9			5	7	1	3														
80	49		3	8	12	6	10	7	5	6		2	1	1										
100	56			5	7	14	11	8	10	8		3	5	2										
120	54				1	6	2	8	5	1		4	3	1	1									
140	40					1	2	5	5			1	3	1		1								
160	18							3	4	1		1	3	1	1									
180	16											1	1	1	1									
200	8																							
220	3																							
240	1																							
260	1																							
280	2																							
293		4	12	25	28	31	35	39	45	23	14	18	6	5	2	1	1	1	1	1	1	1	1	

dem Hungertage wurde Insulin

dem Prae-

Schritt verringert worden, und am Hungertage wurde Insulin erst dann gegeben, wenn Symptome von beginnendem Prækoma, Übelkeit, Brechreiz und Herzklopfen, dazu zwangen.

TAB. 39.

Grenzen, innerhalb welcher der Nüchternblutzuckerwert bei 131 Fällen von O-Diabetes an einem Hungertag schwankte, bevor Insulin wegen beginnenden Praekomas gegeben werden musste.

Nüchtern- Blutzucker- wert mg %	Anzahl Fälle	H ö c h s t e r B l u t z u c k e r w e r t																						
		160	180	200	220	240	260	280	300	320	340	360	380	400	420	440	460	480	500	520	540	560	580	
40	2								1	1														
60	1				1																			
80	2						2																	
100	2	1		1																				
120	2				2																			
140	14			1	3	7	3																	
160	13			2	3	5					1		1	1										
180	20			3	3	4	3	2	2		1		2		1									
200	13				1		7	3		1	1									1				
220	16					3	2	3				4	1	1		1								
240	12						2		3	1	2	3	1	1					1					
260	7							1	1	1	1	3	1	1	1					1				
280	7											1	2						1	1				
300	7											3	1	2										
320	6												3	1					1				1	
340	4													2	2				1					
360	1														1									
380	1																1							
400	1																1							
		131	1		7	13	19	19	9	7	6	9	15	7	6	2	4	1	1	2	2		1	

Die angegebenen Blutzuckerbestimmungen waren *vor* dem Einsetzen des Insulins vorgenommen worden, weshalb ein niedriger Blutzuckerwert nicht eine Folge der Insulinwirkung ist.

Bei einem bedrohlichen Diabetes vom Typus 0 kann der Blutzuckerwert vor dem Praekoma sowohl steigen wie sinken. Die hier folgende Tab. 40 zeigt die Änderung des Blutzuckerwerts, vom Morgen bis Insulin wegen beginnenden Praekomas eingesetzt werden musste, bei 92 Fällen von 0-Diabetes.

Aus der Tab. 40 geht hervor, dass der Blutzuckerwert in etwa derselben Anzahl Fälle beim Beginn des praekomatösen Erbrechens gesunken oder gestiegen war. Veränderungen des Blutzuckerspiegels sind daher nicht eine direkte Ursache für das Zustandekommen des Praekomas. Die Variationen des Blutzuckers im Hunger sind ein Ausdruck der Schwankungen des endogenen Rhythmus, aber das Praekoma entsteht bei zunehmender Ketonkörperbildung. Da eine Kompensationshy-

TAB. 40.

Veränderung des Blutzuckerwerts an einem Hungertag von 8 Uhr bis zum Ausbruch des Praekomas vor Insulingaben bei 92 Fällen von bedrohlichem Diabetes.

Veränderung des Blutzuckergehalts vom Morgenwert bis zum Ausbruch des Praekomas	Anzahl Fälle	
	Steigerung	Senkung
0— 25 mg % .....	17	16
0— 50    »   .....	15	9
0— 75    »   .....	5	8
0—100    »   .....	2	3
0—150    »   .....	1	5
0—200    »   .....	3	4
0—250    »   .....	2	1
0—300    »   .....	—	1
Sa.	45 = 49 %	47 = 51 %

perglykämie der Bildung von Ketonkörpern entgegenwirkt, trägt die Blutzuckersteigerung im Grunde zur Verhinderung des Komas bei. Ein mit den üblichen Blutzuckerbestimmungen (Reduktionsmethoden) erhaltener Wert braucht jedoch dem wirklichen Blutzuckergehalt beim Diabetes nicht zu entsprechen (vgl. S. 286). Die ganze Frage des Blutzuckergehalts und der Ketonkörperbildung ist kompliziert und physiologisch-chemisch noch nicht geklärt.

### Diabetes und Sauerstoffgrundverbrauch

Ein von einer neurohypophysären Störung durch Veränderung der glandotropen Hormonproduktion in der Hypophyse hervorgerufener Diabetes kann leicht auch von Störungen der Schilddrüsenfunktion begleitet sein, wenn gleichzeitig Veränderungen in der Bildung des thyreotropen Hormons vorliegen. Es ist daher von Interesse, zu ermitteln, in welchem Grade der Sauerstoffgrundverbrauch bei den verschiedenen Typen des Diabetes verändert ist. Zu diesem Zweck wurde bei 466 Fällen von Diabetes der Grundumsatz nach Krogh unter Verwendung der Benedict-Harrisschen Normallabellen zur Berechnung des

Grundumsatzwerts bestimmt. Namentlich bei gewissen Diabetesfällen ist der Grundumsatzwert grossen individuellen Variationen unterworfen, auch wenn die Bestimmungen an derselben Tageszeit und unter Beobachtung aller Vorsichtsmassregeln bei der Ausführung der Untersuchungen vorgenommen werden. Die hier folgende Tab. 41 zeigt, wie der Grundumsatzwert bei den verschiedenen Diabetestypen variiert.

TAB. 41.

Grundumsatzwerte bei 466 Fällen von Diabetes ohne Insulin.

Grundumsatzwert • n. Krogh	D i a b e t e s f ä l l e							
	Typus A		Typus B		Typus 0		insgesamt	
	An- zahl	%	An- zahl	%	An- zahl	%	An- zahl	%
<i>Erhöht</i> > + 60 .....	7	3,2	8	6,7	9	6,9	24	5,2
+ 60 .....	5	2,4	10	8,4	15	11,5	30	6,4
+ 40 .....	33	15,3	16	13,4	28	21,4	77	16,5
+ 20 .....	45	20,8	15	12,6	26	19,8	86	18,5
<i>Normal</i> + 10 .....	31	14,4	12	10,1	17	13,0	60	25,5
- 10 .....	28	13,0	17	14,3	14	10,7	59	
- 20 .....	34	15,7	23	19,3	17	13,0	74	15,9
- 40 .....	32	14,8	15	12,6	2	1,5	49	10,5
- 60 .....	1	0,5	3	2,5	3	2,3	7	1,5
<i>Gesenkt</i> > - 60 .....	—	—	—	—	—	—	—	—
Sa. Fälle	216		119		131		466	

Von den Diabetesfällen hatten 27,4 % vom Typus A, 24,4 % vom Typus B und 23,7 % vom Typus 0 normale Grundumsatzwerte, bei den übrigen war der Grundumsatz höher oder niedriger als normal.

### Diabetes und Blutdruck

Bei der Blutzuckerregulation spielt das sympathico-adrenale System eine wichtige Rolle. Da dieses System auch auf Blutdruck und Gefässtonus einen steigernden Einfluss ausübt, liegt

TAB. 42.

Blutdruck bei den einzelnen Diabetestypen in verschiedenen Altersstufen.

Frauen, 565 Fälle.

Alter	Anzahl Fälle	Systolischer Blutdruck, mm Hg											
		60— 80	100	120	140	160	180	200	220	240	260	280	>280
<i>Typus A:</i>													
0—10 J.	6		2		4								
11—20 "	24			10	12	2							
21—30 "	39		1	15	19	4							
31—40 "	27			7	10	4	3	2				1	
41—50 "	25			4	8	9	4						
51—60 "	40			2	9	5	6	8	6	2	2		
61—70 "	33					5	11	11	4	1	1		
71—80 "	6					1	1	2		1	1		
Sa.	200		3	38	62	30	25	23	10	4	4	1	
<i>Typus B:</i>													
0—10 J.	4		1	1	2		1						
11—20 "	11			6	4	—	1						
21—30 "	12			4	6	2							
31—40 "	10			2	3	3	2						
41—50 "	19				8	2	4	3	1	1			
51—60 "	56			1	7	8	15	9	9	4	1	2	
61—70 "	34			1	2	4	7	8	5	2	3	2	
71—80 "	15				2	1	2	5	2	2	1		
81—90 "	1									1			
Sa.	162		1	15	34	20	31	25	17	10	5	4	
<i>Typus 0:</i>													
0—10 J.	9	1	1	5	1	1							
11—20 "	54		2	28	16	5	3						
21—30 "	44			12	22	7	2	1					
31—40 "	34		1	4	19	7	2	1					
41—50 "	26			3	9	8	3	2	—	1			
51—60 "	17			1	2	6	3	—	2	1	—	2	
61—70 "	14				2	4	1	1	2	2	1	1	
71—80 "	4					1	2	1					
81—90 "	1									1			
Sa.	203	1	4	53	71	39	16	6	4	5	1	3	

TAB. 43.

Blutdruck bei den einzelnen Diabetestypen in verschiedenen  
Alterstufen.

Männer, 658 Fälle.

Alter	Anzahl Fälle	Systolischer Blutdruck, mm Hg											
		60— 80	100	120	140	160	180	200	220	240	260	280	>280
<i>Typus A:</i>													
0—10 J.	5			5									
11—20 „	43		3	14	20	6							
21—30 „	52			19	25	5	3						
31—40 „	58			23	17	9	7	1	1				
41—50 „	50			8	18	12	6	3	1	2			
51—60 „	49			4	6	16	16	1	5	1			
61—70 „	28				4	9	7	3	3	2			
71—80 „	5					4				1			
Sa.	290		3	73	90	61	39	8	10	6			
<i>Typus B:</i>													
0—10 J.	1					1							
11—20 „	36		1	12	17	6							
21—30 „	24			7	10	4	3						
31—40 „	23		1	7	9	3	1		1	1			
41—50 „	21			4	8	3	5	1					
51—60 „	35			3	10	11	4	2	2	3			
61—70 „	33			1	6	8	5	5	3	5			
71—80 „	6					3	2	1					
81—90 „	2						1	1					
Sa.	181		2	34	60	39	21	10	6	9			
<i>Typus O:</i>													
0—10 J.	12		1	6	3	1	1						
11—20 „	51		4	17	19	5	4	1	1				
21—30 „	40		1	7	24	6	2						
31—40 „	39			11	17	8	3						
41—50 „	25		1	6	10	6	1	1					
51—60 „	12				3	5	2	2					
61—70 „	7					1	2	2	1	1			
71—80 „	1								1				
Sa.	187		7	47	76	32	15	6	3	1			

es recht nahe, sich die Frage vorzulegen, ob gleichzeitig mit Störungen der Blutzuckerregulation auch Veränderungen der vasomotorischen Regulation mit abnormen Blutdruckwerten vorhanden sind.

Eine Zusammenstellung von 1223 Diabetesfällen, sämtlich ohne Anzeichen einer Nierenschädigung, beleuchtet diese Frage. Das Material enthält 673 Männer und 549 Frauen. Unter den Männern waren 290 Diabetesfälle vom Typus A, 181 vom Typus B und 187 vom Typus 0. Bei den Frauen war die Zusammensetzung folgende: 200 Diabetesfälle vom Typus A, 162 vom Typus B und 203 vom Typus 0.

In den vorstehenden Tabellen 42 und 43 findet man die oberen Grenzwerte des systolischen Blutdrucks bei den verschiedenen Diabetestypen. Die Patienten sind nach Altersgruppen geordnet. Der Blutdruck wurde stets bei Bettruhe und wiederholt gemessen, weshalb zufällige Blutdrucksteigerungen ausgeschlossen sind.

## Diabeteskomplikationen

Der Diabetes geht nicht selten mit anderen krankhaften Zuständen Hand in Hand. Es ist daher grundsätzlich wichtig, zu ergründen, ob die komplizierende Erkrankung eine Folge der diabetischen Stoffwechselstörung oder nur ein von dieser unabhängiges Parallelphänomen ist.

Bei Störungen der glandotropen Hormonbildung der Hypophyse kann ein Diabetes vom hypophysären Typus auftreten. Dieser kann da auch von anderen krankhaften Zuständen begleitet sein, z. B. von Hyperthyreose, Störung der Geschlechtsfunktionen, abnormer Fettbildung usw. Diese Zustände sind selbstverständlich keine Folgen der vorhandenen diabetischen Stoffwechselstörung, sondern von dieser unabhängige Parallelerscheinungen, nur von einer gemeinsamen Ursache, in diesem Falle der gestörten glandotropen Hypophysenfunktion, ausgelöst. Ein diagnostizierter Diabetes stellt da nur ein Teilsymptom in der endokrinen Störung dar.

## Diabetes und Augenveränderungen

Unter den Komplikationen, welche den Diabetes begleiten, haben die Augenveränderungen schon früh Beachtung gefunden. Als recht spezifisch für Diabetes mellitus gelten Star und Retinitis.

Das Studium der Augenveränderungen beim Diabetes besitzt spezielles Interesse, da die ophthalmologische Untersuchung durch die direkte Betrachtung krankhafter Veränderungen besondere Möglichkeiten für eine exakte Diagnostik und für die Beurteilung der Bedeutung des diabetischen Zustands für die Entwicklung der Augenerkrankungen schafft. Die Schwierigkeit ist, diabetische Augenveränderungen von solchen anderen Ursprungs abzugrenzen. Deshalb schwanken auch die Angaben im Schrifttum.

Neben Katarakten und Retinitiden diabetischen Charakters gibt es bei Diabetikern noch andere Augensymptome ätiologisch zweifelhafteren Ursprungs, Chorioiditiden, Iritiden, Konjunktivitiden, Epitheldegeneration, senile Veränderungen, Refraktionsanomalien von permanenter oder transitorischer Art u. a. m., bei welchen in vielen Fällen ein bestehender Diabetes keine Bedeutung zu haben braucht. Diese können daher in einer gemeinsamen Gruppe zusammengefasst werden.

Mein eigenes Material von Augenkomplicationen enthält 865 Diabetesfälle der verschiedenen Typen, sämtlich ohne Anzeichen einer Nierenschädigung und von einem erfahrenen Augenarzt eingehend beobachtet. Eine Gruppierung der Augenkomplicationen unter Berücksichtigung der Diabetestypen folgt in Tab. 44.

Bemerkenswert ist die relativ hohe Frequenz von Retinitiden beim Diabetes vom Typus B. Da es die Gruppe B-Diabetes ist, in welcher die insulinresistenten Fälle angetroffen werden, kann dies mit der Beobachtung von *Falta* gut im Einklang stehen, dass Retinitiden bei insulinresistenten Diabetesfällen häufiger sind.



TAB. 44.  
Augenkomplikationen bei 865 ophthalmologisch untersuchten  
Fällen von Diabetes.

Diabetestypus	Fälle	Komplikationen					
		Katarakt		Retinitis		Sonstige Veränderungen	
		Fälle	%	Fälle	%	Fälle	%
Typus A .....	398	6	1,5	11	2,8	14	3,5
Typus B .....	245	6	2,5	20	8,2	9	3,7
Typus 0 .....	222	7	3,2	6	2,7	8	3,6
Sa.	865						

### Diabetes und Arteriosklerose

Beim Diabetes ist die Häufigkeit der Arteriosklerose eine grosse (*Blotner, Bowen und König, Joslin und White, Nathanson, Rabinowitch, Warren u. a.*). *V. Noorden* betont, dass die Arteriosklerose kein obligater Begleiter des Diabetes sei. Dieselbe ist bei leichten Diabetesfällen häufiger, bei den schwersten seltener. Vor der Einführung des Insulins erreichten die schweren Diabetesfälle kein so hohes Alter, dass sich arteriosklerotische Symptome nachweisen liessen. Eine essentielle Hypertonie ist nicht der Ausdruck arteriosklerotischer Gefässveränderungen, weshalb Hypertonie beim Diabetes nicht gleichwertig mit Arteriosklerose ist.

Mit der Einführung der Insulinbehandlung und der hierdurch bedingten längeren Lebensdauer der schweren Diabetesfälle hat die Arteriosklerose auch unter diesen zugenommen.

Bei der Beurteilung der Frage des Auftretens der Arteriosklerose bei Diabetikern muss man zwischen der Wirkung der diabetischen Stoffwechselstörung und anderen die Arteriosklerose begünstigenden Faktoren, wie Tabakgenuss, Alkoholismus und Lues, unterscheiden. Einem erhöhten Cholesterin-gehalt des Blutes beim Diabetes ist von einigen Autoren (z. B. von *Joslin*) eine gewisse Bedeutung für die Entstehung der Arteriosklerose zugeschrieben worden, was aber von anderen

(Falta) bestritten wird. Die Rolle des Cholesterins im Stoffwechsel ist unbekannt.

Der Zusammenhang zwischen Diabetes und Arteriosklerose ist nicht klar. Eine hohe Arteriosklerosefrequenz beim Diabetes braucht nicht unbedingt zu bedeuten, dass eine diabetische Stoffwechselstörung die Ursache der Arteriosklerose ist. Bei beginnender Arteriosklerose kann es zu einer Erschwerung des Stoffaustausches zwischen Zellen und Geweben infolge von Veränderungen der Permeabilität des Gefäßsystems kommen. Dadurch können auch Störungen der biologischen Oxydationsprozesse in Organen und Geweben eintreten, mit einer Gewebsanaerobie, die zu einem Asphyxiadiabetes führen kann. Der diabetische Zustand ist in diesem Falle eine Folge der Arteriosklerose. Es ist da ganz natürlich, dass die Arteriosklerosefrequenz beim Diabetes hoch sein muss, aber Diabetes- und Arterioskleroseproblem sind dabei auf ganz andere Weise miteinander verknüpft, als wenn die Arteriosklerose eine Folge eines vorliegenden Diabetes wäre.

Die Frage des Zusammenhangs zwischen Arteriosklerose und Diabetes ist indessen noch verwickelter. Man muss nämlich auch mit der Möglichkeit rechnen, dass beim Diabetes unvollständig abgebaute Stoffwechselprodukte mit deletärem Einfluss auf Gefäße und Gefäßwände auftreten können, deren Wirkung man nicht kennt. Ein erhöhter Blutzuckerwert kann dabei eine grundsätzlich verschiedene Bedeutung erhalten, wenn eine Kompensationshyperglykämie vorliegt, oder wenn derselbe auf einer Anhäufung von reduzierenden, unvollständig umgesetzten Kohlehydratstoffwechselprodukten beruht (vgl. S. 287).

Treten bei einer Störung im Kohlehydratstoffwechsel anaerobe Abbauprodukte in gesteigerter Menge auf, so brauchen diese den Reduktionswert des Blutes nicht zu beeinflussen. Sie bringen da auch den Blutzuckerwert nicht zum Steigen. Trotzdem können diese Abbauprodukte Schädigungen des Gefäßsystems verursachen, analog der prädisponierenden Wirkung des Alkohols zu arteriosklerotischen und degenerativen Gefäßveränderungen. Da eine abnorme Alkoholbildung der Ausdruck einer Anaerobie im Organismus ist, tritt diese ebenso-

wohl im Hunger wie bei Nahrungszufuhr auf (vgl. S. 318). *Karlström* fand bei einer Untersuchung diabetischer Kinder, welche fünf Jahre lang eine freie gemischte Normalkost erhalten hatten, auch keinerlei Anzeichen von Arteriosklerose oder anderen pathologischen Veränderungen infolge der gesteigerten Nahrungszufuhr. Ein zur Klärung der Frage der Beziehung zwischen Arteriosklerose und Diabetes — ob irgend ein Kausalzusammenhang besteht und *was* in diesem Falle das Primäre ist, der arteriosklerotische Prozess oder die diabetische Stoffwechselstörung — geeignetes Material steht zur Zeit noch aus.

Was für die Arteriosklerose gilt, das gilt in noch höherem Grade für die *diabetische Gangrän*. Bei Personen in höherem Lebensalter ist Gangrän an den unteren Extremitäten als eine Folge arteriosklerotischer Gefäßveränderungen auch ohne Diabetes keine seltene Erscheinung.

Infolge der längeren Lebensdauer der Diabetiker nach Einführung der Insulinbehandlung ist auch die Gangränfrequenz beim Diabetes gestiegen. Die Insulintherapie scheint dabei keinen Schutz vor der Entwicklung der Arteriosklerose gewährt zu haben. Nach *Strömbeck* stieg die Anzahl der in der chirurgischen Abteilung des Serafimerlazarets in Stockholm beobachteten Fälle von diabetischer Gangrän nach der Einführung des Insulins im Jahre 1923 von im Durchschnitt 2,6 auf 4,4 pro Jahr. Die entsprechende Frequenz seniler Gangrän ohne Diabetes war durchschnittlich 2,6 Fälle pro Jahr vor bzw. 3,3 nach dem Jahre 1923.

Die diabetische Gangrän kann auf verschiedene Weise entstehen.

Bei einem Teil der Fälle handelt es sich um reine *arteriosklerotische Veränderungen von obliterierender Natur*, evtl. mit Arterienthrombosen in Gefäßen der unteren Extremitäten. Nur wenn die Arteriosklerose eine Folge der diabetischen Stoffwechselstörung bildet, ist eine Gangrän dieses Typs von einem vorhandenen Diabetes verursacht. Ist dies nicht der Fall, dann besteht kein Zusammenhang zwischen dem Diabetes und der Entstehung der Gangrän. Dieselbe ist eine arteriosklerotische Gangrän, welche einen Diabetiker betroffen hat.

Bei anderen Fällen ist die Gangrän eine Folge von *spastischen Gefäßveränderungen* oder *trophischen Störungen nervöser Art*. Auch in diesem Fall kann die Gangrän eine Parallelerscheinung eines vorhandenen Diabetes sein, und beide können durch einen abnormen Reizzustand im vegetativen bzw. vasomotorischen Nervensystems verursacht sein (vgl. S. 8 u. 190).

Der Entstehungsweise des Brandes ungeachtet muss man zwischen der primären Ursache desselben und der sekundären Komplikation beim Hinzutreten einer Infektion streng unterscheiden. Bei einer Infektion erfolgt in der Regel eine Verschlimmerung des diabetischen Zustands, mit gesteigerter Azidose, wodurch der Zustand noch schlechter, die Widerstandsfähigkeit der Gewebe verringert und dem Fortschreiten der Gangrän der Weg geebnet wird. Eine energische und effektive Insulinbehandlung kann dabei die sekundären Symptome zum Zurückgehen bringen, aber die primäre Ursache des Brandes bleibt bestehen, und chirurgische Hilfe kann erforderlich werden.

Als Beispiele für die Entwicklung eines Diabetes mit Gangrän werden folgende sieben Krankengeschichten angeführt. Zu einer statistischen Klärung der Frage der Beziehungen zwischen Diabetes, Arteriosklerose und Gangrän ist ein grösseres, in allen Punkten durchgearbeitetes Material erforderlich.

*Fall 30.* Alfred A., geb. 1867.

1921 leichte Gehirnthrombose, welche sich 1931 wiederholte. Seit 1930 zeitweise Schmerzen in den Beinen, besonders im rechten. In dieser Zeit wurde im Zusammenhang mit einem infizierten Clavus am rechten Fuss ein leichter Diabetes entdeckt. Strenge Diät (Fett-Gemüsekost + 70 g Brot) während dreier Jahre. Im November 1933 beginnende Gangrän des rechten Fusses, und im Juni 1934 musste das rechte Bein hoch amputiert werden.

Diabetes vom Typus B. Blutdruck 200/100.

*Pathologisch-anatomischer Befund:* Hochgradige Arteriosklerose der Gefässe des Beins mit fast vollständiger Obliteration der Art. poplitea.

*Fall 31.* Ellen F., geb. 1866.

Seit 1915 Arthritis-deformansbeschwerden im rechten Knie und der rechten Schulter. Herzbeschwerden mit Kurzatmigkeit und Anschwellung der Beine seit 1923. 1931 wurde zufällig Zucker im Urin

konstatiert, im Frühjahr 1932 Gewichtsabnahme von 87 auf 81 kg. Länge 158 cm. Blutdruck 230/100.

Diabetes vom Typus B. Mässige Diät (100—150 g Brot + 100 g Kartoffeln). Ziemlich munter bis Februar 1940, da beginnende Gangrän des rechten Fusses, im März d. J. hohe Amputation des rechten Beines.

*Pathologisch-anatomischer Befund:* Hochgradige Arteriosklerose, namentlich der Art. poplitea.

*Fall 32.* Harald J., geb. 1881.

1925 wurde bei einer Untersuchung für Lebensversicherung zufällig Zucker festgestellt. Nach einigen Tagen leichter Diät verschwand der Zucker, dann keine anderen Symptome. Seit 1936 leichte Herzbeschwerden mit praekordialen Schmerzen und Kurzatmigkeit bei Anstrengungen. Im Juli 1939 Hautabschürfung durch Schuhdruck, Ostitis und Sequestrierung der linken kleinen Zehe. Im Januar 1940 geheilt. Am August 1940 infizierter Clavus an der rechten kleinen Zehe. Im Anschluss daran entwickelte sich eine Gangrän des rechten Fusses und Unterschenkels. Das rechte Bein musste im Oktober 1940 über den Knie amputiert werden. Blutdruck 140/80. Leichte Kardiosklerose.

Leichter Diabetes vom Typus B.

*Pathologisch-anatomischer Befund:* Ausgesprochene arteriosklerotische Veränderungen, aber dazu Anzeichen degenerativer Nervenveränderungen mit Zerfall der Markscheide peripherer Nerven.

*Fall 33.* Gustav A., geb. 1885.

Früher stets gesund gewesen. Der Diabetes wurde 1923 konstatiert. Mässig strenge Diät. Seit Herbst 1931 ziehende Schmerzen in den Füßen. Blutdruck 145/85.

Diabetes vom Typus A. Röntgenbefund: Ausgesprochene Gefässverkalkung in den Arterien des Unterschenkels und Fusses, namentlich in den Metatarsalgefässen. Wa. R. neg. Cardiosclerosis levis. Seit 1934 zunehmende Parästhesien in den Füßen mit herabgesetzter Sensibilität an den Füßen und Unterschenkeln. Im Dezember 1939 beginnende Gangrän des linken Beines, im Januar 1940 Amputation über dem linken Knie.

*Pathologisch-anatomischer Befund:* Starke arteriosklerotische Veränderungen in den Gefässen und degenerative Veränderungen peripherer Nerven.

*Fall 34.* James L., geb. 1883.

1921 Gallensteinanfall. Im Zusammenhang damit zeitweise etwas Durstgefühl. Im Sommer 1922 wurde ein leichter Diabetes entdeckt. Mässig strenge Diät. Seit Frühjahr 1934 Parästhesien in den Füßen

und Zehen. Hochgradige Gefäßverkalkung in den Füßen und Unterschenkeln. Wa.R. neg. Blutdruck 160/90. Kardiosklerose und Retinitis vom diabetischen Typus.

Diabetes vom Typus B. Im Frühjahr 1936 beginnende Gangrän der 1. und 2. Zehe des linken Fusses. Im September 1936 Amputation des vorderen Teils des linken Fusses bis zur Hälfte der Metatarsalknochen. Trotz hochgradiger Gefäßverkalkung Sekundärheilung ohne Rezidiv. Mit Insulin (32 + 32 I.E.) ging es Pat. dann gut bis Oktober 1942, wo er plötzlich an Haemorrhagia cerebri ad exitum kam. Der Fall ist vom Standpunkt des Chirurgen von Strömbeck beschrieben worden.

*Fall 35. Carl T., geb. 1883.*

Vater an Diabetes gestorben. Pat. leidet von Kindheit an unter Blutwallungen nach dem Kopf. 1900 wurde zufällig Zucker im Urin konstatiert; der Zucker verschwand nach kurzer Zeit. Sonst gesund. 1917 quälendes Durstgefühl, wobei der Diabetes festgestellt wurde. Seit dieser Zeit sehr strenge Diät, zeitweise ohne Brot und Kartoffeln. Im Herbst 1910 Herzbeschwerden mit präkordialen Schmerzen und Anschwellung der Beine. Im Januar 1911 zunehmende Parästhesien im linken Bein. 28. III. 41 Blutdruck 215—170/90; Kardiosklerose und ausgedehnte arteriosklerotische Gefäßveränderungen. Neurologische Untersuchung: Keine Sensibilitäts- oder Reflexstörungen.

Leichter Diabetes vom Typus B. Die Insulinbehandlung wurde auf Grund relativer Indikation mit 24 + 20 I.E. eingeleitet. Trotzdem nahmen die Beschwerden im linken Bein zu. Im November 1911 beginnende Gangrän des linken Fusses, welche trotz intensiverer Insulinbehandlung, Priscol, Azetylcholin und Nitroglyzerin, Pavexbehandlung u. a. m. Fortschritte machte und im Dezember d. J. zur Amputation des linken Beinen über dem Knie zwang.

*Pathologisch-anatomischer Befund:* Hochgradige, teilweise obliterierende Gefäßverkalkungen, Venenthrombosen und Nervendegeneration in N. tibialis und Nn. peronaei (superficialis et profundus).

*Fall 36. Marie A., geb. 1895.*

Beide Eltern Diabetiker. 1910 Basedow, Jodbehandlung ohne Operation, nach ca. 6 Monaten wiederhergestellt. 1915 im Zusammenhang mit Gravidität zunehmende Korpulenz, das Gewicht stieg auf 120 kg (1925). Wiederholte Entfettungskuren in Marienbad. Seit 1939 Schwindel, Kopfschmerzen, zeitweise Angstgefühl und Unruhe, Parästhesien in Fingern und Zehen, mitunter Anschwellung der Finger. Schlaf schlecht. Im März 1942 Blasenbildung an der rechten

grossen Zehe, mit Schwellung und Exulzeration. 27. V. 42 Gewicht 94,2 kg, Länge 165 cm. Blutdruck 230/120, Pulsfrequenz 120.

Leichter Diabetes vom Typus B. Rechte grosse Zehe kolbenförmig angeschwollen, sequestrierende Ostitis der Endphalanx mit infiziertem Geschwür medial. Nach Ausschabung des ostitischen Herdes geheilt. Im August 1942 gangränöses Ulcus am vorderen Teil der rechten Fusssohle, anfangs abgegrenzt, dann aber trotz forcierter Insulinbehandlung und gefässerweiternder Mittel um sich greifend; es kam eine putride Infektion hinzu, weshalb im Oktober d. Jahr die Amputation des rechten Fusses und unteren Drittels des Unterschenkels auf Grund vitaler Indikation vorgenommen werden musste.

*Pathologisch-anatomischer Befund:* Leichte arteriosklerotische Gefässveränderungen, aber mikroskopisch mehr oder minder ausgesprochene Nervendegeneration mit Zerfall der Markscheide in peripheren Teilen.

In bezug auf *Herzkomplikationen* beim Diabetes gilt weitgehend das bei der Arteriosklerose Gesagte. Hier ist es von besonderer Bedeutung, zwischen Ursache und Wirkung zu unterscheiden, da die Behandlung sonst leicht auf Abwege geraten kann (vgl. Fall 7 S. 270).

Ein sicherer ursächlicher Zusammenhang zwischen Diabetes und Herzveränderungen besteht beim bedrohlichen Diabetes, wenn die Ketonkörperbildung steigt und ein Praekoma eintritt. Dabei sind bisweilen Veränderungen des Elektrokardiogramms zu beobachten, welche auf eine gestörte Herzfunktion hindeuten. Diese ist eine Folge einmal der Verschiebung des physikalisch-chemischen Regulationsgleichgewichts im Zusammenhang mit der Azidose und ferner der herabgesetzten Kohlehydratverwertung mit ungenügender Glykogensynthese. Diese Herzveränderungen sind transitorisch und verschwinden, wenn die Ketonkörperbildung durch die Insulinwirkung aufhört, die Azidose verschwindet und der Kohlehydratstoffwechsel besser wird. Wenn dagegen die Ketonkörperbildung nicht zum Stehen kommt, sondern der komatöse Zustand Fortschritte macht, kann die daniederliegende Herzfunktion dem Kranken zum Verhängnis werden und den Tod infolge von Herzkollaps verursachen. Infolgedessen muss bei hochgradiger Azidose und diabetischem Koma der Zustand des Herzens stets aufs sorgfältigste beachtet werden.

## IX. ABSCHNITT

Der endogene Rhythmus des Stoffwechsels  
und die Diabetesbehandlung.

## Diabetes und Diät

Das erste Erfordernis für eine erfolgreiche Diabetesbehandlung ist ein möglichst klarer Einblick in das Wesen des diabetischen Zustands. Liegt als beitragender Faktor bei der Stoffwechselstörung ein Mangelzustand vor, so ist zuerst dieser nach Möglichkeit zu beseitigen. Ohne Rücksicht hierauf zu versuchen, durch Nahrungsbeschränkung oder Kohlehydratenziehung Blutzuckergehalt und Zuckerdiurese herabzudrücken, mit der Absicht, dadurch einen vorliegenden Diabetes zu bessern, das ist keine rationelle Therapie. Bei Diabetesformen vom Typus A wird die Stoffwechselstörung hierdurch nur schlimmer: die Glykogenbildung geht zurück und die Ketonkörperproduktion nimmt zu, je grösser der Kohlehydratmangel im Organismus wird.

Sehr lehrreich ist ein näheres Studium des Verhaltens des Blutzuckers beim Diabetes in Tab. 19 S. 288 (Fall 1 u. 2). Die Tabelle stammt von vier Diabetesfällen, zwei vom Typus A und zwei vom Typus B. In derselben Blutprobe ist hier der Blutzuckergehalt einerseits mit der gewöhnlichen Hagedorn-Jensenschen Reduktionsmethode und andererseits mit einem für das Hexosemolekül spezifischen Verfahren, der *Orzinmethode* (vgl. S. 97 u. 287), bestimmt. Aus der Tabelle geht hervor, dass der erste Diabetesfall vom Typus A bei Bestimmung nach Hagedorn-Jensen während eines Hungertages normale oder subnormale Blutzuckerwerte aufweist. Die Blutzuckerbestimmung mit der Orzinmethode zeigt dagegen, dass Glykose im Blut bei Hunger vollständig fehlt. Bei Nahrungszu-



fuhr steigt der Hexosengehalt des Blutes. Wenn der Orzinwert höher ist als der entsprechende Reduktionswert, müssen die Hexosen zum Teil in polymerisierter oder aldehydgruppenblockierter Form vorhanden sein.

Bei dem zweiten Fall von A-Diabetes in der Tabelle ist der Reduktionswert des Blutzuckers dagegen erhöht. Trotz des Hungers ist hier der Orzinwert erheblich höher. Bereits im Hunger findet sich also hier ein bedeutender Kohlehydratüberschuss im Blute, welcher nicht normal umgesetzt wird. Erhält in diesem Falle der Patient eine gewöhnliche, gemischte freie Kost, so steigt zwar der Reduktionswert des Blutzuckers, der Orzinwert hingegen sinkt und wird sogar an ein paar Zeitpunkten null. Dies bedeutet, dass sich der Kohlehydratstoffwechsel trotz der steigenden Reduktionswerte gebessert hat, was sich auch in einer geringeren Ketonkörperausscheidung zeigt.

Bei diesen beiden Diabetesfällen vom Typus A mit extremen Veränderungen des Zuckergehalts des Blutes ist daher die Verabreichung einer normalen, gemischten und genügend kohlehydratreichen Nahrung in mässiger Menge eine völlig rationelle Therapie. Hunger und Kohlehydratentziehung verursachen bei diesen Fällen eine Verschlechterung des Kohlehydratumsatzes, wodurch die Stoffwechselstörung als Ganzes verschlimmert und die Schädigung des Fetthaushalts grösser wird. Dies zeigt sich u. a. in einer gesteigerten Ketonkörper- und Cholinausscheidung im Hunger, ein Zeichen eines beeinträchtigten Lipoidstoffwechsels (s. Tab. 28, S. 318). *Mit Hunger lässt sich dementsprechend in diesen Fällen nichts erreichen, auch wenn die Zuckerdiurese verschwindet.*

Ganz anders liegen dagegen die Dinge bei den beiden Diabetesfällen Typus B (Fall 3 u. 4, Tab. 20 S. 288). Hier ist die Diskrepanz zwischen Reduktions- und Orzinwert des Blutzuckers durchweg nicht so gross. Ein Hexosemangel im Blute liegt bei diesen Fällen selbst im Hunger nicht vor. Die basale Blutzuckerregulation genügt in diesen Fällen vollständig dazu, dass der Blutzuckerbedarf des Organismus gedeckt wird. Bei Nahrungszufuhr hingegen werden die Kohlehydrate nicht in normaler Weise abgebaut, sie häufen sich daher im Blute an.



TAB. 45.

Mittel der höchsten und niedrigsten von fünf während des Tages beobachteten Blutzuckerwerten bei 30 Fällen von Diabetes im Hunger ohne Insulin und bei Nahrungszufuhr.

	H u n g e r				Gewöhnliche Kost			
	Blutzucker		Urin 8—20 Uhr		Blutzucker		Urin 8—20 Uhr	
	Hagedorn	Orzin	Zucker	$\beta$ -Oxy- butter- säure	Hagedorn	Orzin	Zucker	$\beta$ -Oxy- butter- säure
	mg %	mg %	g	mg	mg %	mg %	g	mg
<i>Mit Ketonurie:</i>								
Olov A.	87 ± 27	48 ± 32	0,9	35	231 ± 52	162 ± 53	79,6	0 I <sup>1</sup>
Göran W.	100 ± 42	54 ± 34	0	369	326 ± 38	247 ± 44	111,7	0 I
Börje G.	107 ± 42	90 ± 38	0,4	93	403 ± 132	303 ± 83	87,7	0 I
Karin P.	116 ± 25	59 ± 22	0	54	246 ± 145	175 ± 95	18,3	0 —
Ulf N.	135 ± 29	83 ± 23	4,9	2923	351 ± 89	223 ± 77	53,5	811 I
Anna H.	145 ± 25	184 ± 67	9,2	48	240 ± 42	453 ± 113	80,3	0 —
Sture Å.	150 ± 42	101 ± 38	5,1	178	260 ± 82	210 ± 66	82,5	0 I
Lisa H.	155 ± 37	123 ± 34	0,5	88	350 ± 47	247 ± 52	104,3	0 —
Fredrik S.	159 ± 93	174 ± 40	0	178	359 ± 82	291 ± 49	49,4	0 —
Ann-Marie W.	163 ± 35	112 ± 25	27,5	2838	338 ± 75	242 ± 63	257,5	0 I
Gösta N.	175 ± 28	148 ± 51	18,2	1144	253 ± 93	159 ± 76	105,3	602 —
Hildur A.	218 ± 49	161 ± 45	6,9	1023	350 ± 58	240 ± 41	72,6	0 I
Erik F.	233 ± 24	209 ± 34	27,4	528	299 ± 76	225 ± 62	129,4	0 I
Ingmar Ö.	249 ± 35	149 ± 43	17,5	153	383 ± 61	315 ± 75	165,9	0 I
Halvard L.	252 ± 79	197 ± 73	33,7	1330	273 ± 100	147 ± 91	39,3	0 I
Ola T.	259 ± 152	211 ± 142	0	194	270 ± 131	234 ± 119	39,7	0 I
Ebba K.	298 ± 49	135 ± 38	27,7	8852	359 ± 93	299 ± 85	121,3	0 I
Åke E.	320 ± 213	192 ± 130	34,5	2198	247 ± 109	224 ± 116	60,6	0 I
<i>Ohne Ketonurie:</i>								
Arvid H.	118 ± 25	75 ± 19	0,8	0	239 ± 54	170 ± 49	38,4	0 —
Anna N.	122 ± 24	70 ± 20	0	0	108 ± 23	70 ± 24	0	0 —
Rara L.	125 ± 38	70 ± 29	2,6	0	201 ± 24	146 ± 28	0,4	0 —
Gunnar O.	162 ± 45	193 ± 37	6,6	0	262 ± 35	174 ± 35	61,0	0 I
Einar Å.	169 ± 41	146 ± 55	0,7	0	330 ± 61	271 ± 47	79,9	0 I
Selma S.	176 ± 33	139 ± 37	0	0	345 ± 62	277 ± 41	12,8	0 —
Ingeborg S.	181 ± 45	97 ± 48	5,8	0	276 ± 14	179 ± 30	65,2	0 —
Gerda A.	191 ± 80	108 ± 84	2,7	0	248 ± 92	200 ± 79	54,3	0 I
Hilda S.	200 ± 75	164 ± 94	0,8	0	260 ± 62	215 ± 38	18,4	0 —
Holger H.	212 ± 35	153 ± 53	3,0	0	334 ± 101	233 ± 87	37,6	0 I
Britta S.	220 ± 90	131 ± 82	0,28	0	172 ± 44	82 ± 49	18,9	0 —
Gunnar K.	255 ± 73	162 ± 68	18,5	0	322 ± 107	226 ± 75	148,4	0 —

<sup>1</sup> Gewöhl. Kost + Insulin.



stellt da eine »Lücke« im Zitronensäurezyklus dar, wodurch die beim Diabetes sowieso schon herabgesetzten Stoffwechselvorgänge fernerhin in Mitleidenschaft gezogen werden. Namentlich bei Diabetesformen mit Störungen des Zitronensäureumsatzes kann aus diesem Grunde eine Beschränkung des Eiweissgehalts der Nahrung angebracht sein. Es ist zur Zeit nicht möglich, eine optimale Eiweissmenge anzugeben. Bei einer basenreichen Kost darf die Eiweisszufuhr nicht zu gross sein, um die oxydativen Stoffwechselprozesse nicht unnötig zu belasten. In diesem Punkte unterscheidet sich die Kost des Diabetikers durchaus nicht von der auch für nicht Zuckerkrankte rationellen Ernährung.

Die Zeiten für die Nahrungsaufnahme sind unter Berücksichtigung der Assimilationsperioden des Organismus festzulegen: Hauptmahlzeit mitten am Tage, leichtere Mahlzeiten morgens und abends (vgl. S. 75 u. 273).

Das Verhalten der Zuckerdiurese und des Körpergewichts gibt bei der Beurteilung des Ernährungszustandes und der Kontrolle der Nahrungsmenge wertvolle Fingerzeige. Dies gilt auch bei einer richtig geleiteten Insulintherapie.

## Insulinbehandlung und endogener Rhythmus

Ein auf mangelhafter Insulinwirkung beruhender Diabetes erfordert Insulinbehandlung zur Besserung der Stoffwechselstörung. *Die Ketonkörperbildung ist dabei in den meisten Fällen ein wichtiges Kriterium für den Insulinmangel (s. S. 275).* Auf Grund der periodischen Ketonkörperbildung im Zusammenhang mit der rhythmischen Lebertätigkeit muss die Insulinbehandlung der periodischen Tätigkeit der Leber angepasst werden. Ausscheidungsdiagramme bilden infolgedessen eine gute Richtschnur für eine rationelle Insulinbehandlung.

Bei ketonkörperbildenden Diabetesformen mit periodischer  $\beta$ -Oxybuttersäureausscheidung spiegeln die Ketosäurewellen im Ausscheidungsdiagramm die rhythmische Lebertätigkeit direkt wider. Die Insulindosierung soll da mit Rücksicht auf die  $\beta$ -

Oxybuttersäurewellen der Ausscheidungsdiagramme festgesetzt werden, so dass die Ketonkörperbildung aufhört. Da die direkte Insulinwirkung etwa vier Stunden anhält (vgl. S. 86) und die Entwicklung der  $\beta$ -Oxybuttersäurewellen ungefähr dieselbe Zeit in Anspruch nimmt, müssen bei einer lege artis geleiteten Insulintherapie diese Zeitperioden soweit möglich zusammenfallen. Ist die Ketonkörperbildung bereits in Gang gekommen, bevor das Insulin zu wirken anfängt, so verschwindet die Ketonurie weniger leicht und kann weiterbestehen, auch wenn die Insulinwirkung zeitweise so stark wird, dass hypoglykämische Beschwerden auftreten. Wenn die Insulindosis da auf einen geeigneten Zeitpunkt verschoben wird, verschwindet die Ketonkörperausscheidung sehr rasch.

Der folgende Fall ist ein Beispiel für bedrohlichen Diabetes, bei welchem die Insulinbehandlung schon von vornherein unter Berücksichtigung des endogenen Rhythmus eingeleitet wurde:

Fall 37. Asta A., geb. 1916

Ein Vetter der Mutter leidet an Diabetes, sonst nichts als erbliche Diabetesveranlagung bekannt. Blutermitt 1928. Leidet seit mehreren Jahren an Migräne; die Anfälle können 2–3 Tage anhalten.

Sonst stets gesund gewesen, erkrankte Ende November 1933 unter zunehmendem Durst, Herabsetzung des Schvermögens und Abmagerung trotz schärfsten Appetits. Mitte Dezember d. J. immer stärker werdende Kurzatmigkeit und Herzbeschwerden. Die Patientin suchte da einen Arzt auf, welcher den Diabetes feststellte. 19. XII. 1933 Gewicht 51,7 kg bei einer Länge von 172 cm. Blutdruck 155/80. Augenhintergrund beiderseits und innere Organe o. B. Die hier eingefügte Abbildung 93 zeigt das Ausscheidungsdiagramm an zwei insulinfreien Tagen, von welchen der eine ein Hungertag war. An diesem verschwindet die Zuckerausscheidung, aber es treten zwei deutliche endogene Ketsäurewellen um 8 bzw. 16 Uhr hervor. Nach Einsetzen der Insulindosen an diesen Zeiten verschwand bei einer freien, gemischten Normalkost bald jede Spur von Ketonurie. Die Zuckerausscheidung bestand noch einige Zeit weiter, war aber Mitte Januar bei einer 32 ± 32 I. E. entsprechenden Insulindosis nicht länger vorhanden; die Insulindosis musste da herabgesetzt werden, um Insulinbeschwerden zu vermeiden, und zwar auf 21 ± 21 I. E., nach wie vor bei einer freien gemischten Kost. Das Gewicht war inzwischen auf 58,5 kg gestiegen. Die Patientin ist seitdem bei letztgenannter Insulindosis gesund und voll arbeitsfähig gewesen. Bei

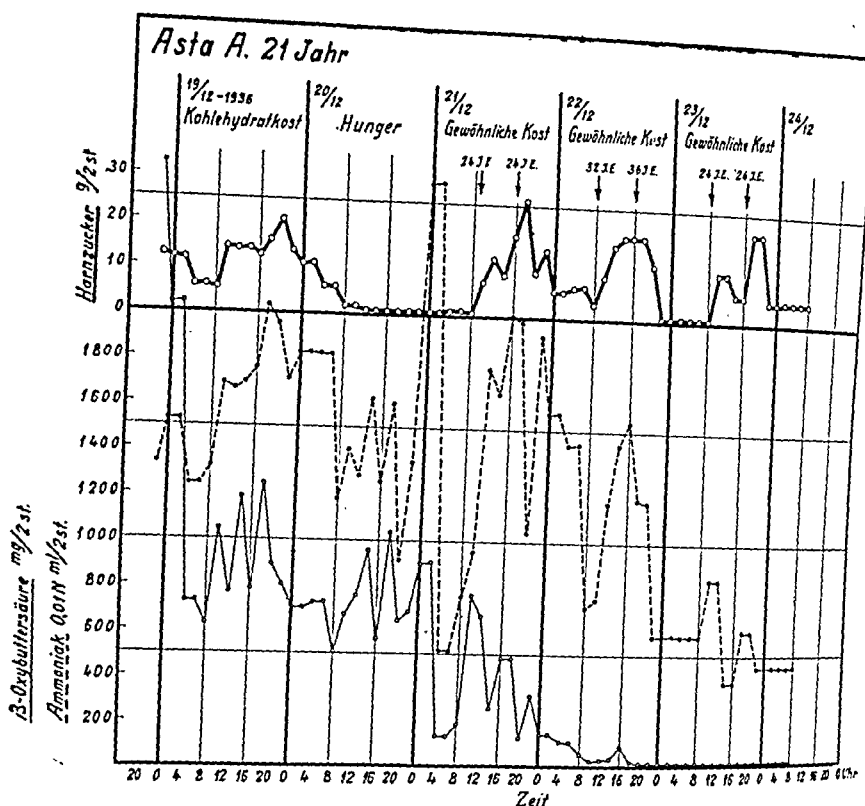


Abb. 93. Ausscheidungsdiagramm bei frischem ketonkörperbildendem Diabetes. Zu beachten sind zwei deutlich markierte endogene  $\beta$ -Oxybuttersäureausscheidungswellen während des Hungertages bei verschwindender Zuckerdiurese. Wenn das Insulin an dem Zeitpunkt des Tages gegeben wird, an welchem sich diese  $\beta$ -Oxybuttersäurewellen zu entwickeln beginnen, verschwindet die Ketonurie spurlos, aber die Zuckerausscheidung besteht bei vermehrter Kost noch einige Zeit weiter.

einer Anfrage im Sommer 1939 wurde die Frage nach dem Allgemeinzustand folgendermassen beantwortet:

»Seit der Behandlung im Dezember 1936 mit Insulineinstellung geht es mir viel besser, und auch die Kopfschmerzen sind jetzt verschwunden. Meine Dankbarkeit für meine gute Gesundheit ist gross. Bin zur Zeit in einer grösseren Hutfabrik angestellt, und es gefällt mir dort wegen uninteressanter Arbeit vielleicht nicht so gut, halte aber desto grössere Anstrengungen aus. Bin unbeschreiblich froh über die mir zuteil gewordene Hilfe.»

Im Gegensatz zu dem vorstehenden Fall, bei dem die Insulindosen von vornherein dem endogenen Rhythmus richtig angepasst wurden, bietet der folgende ein Beispiel für bedrohlichen Diabetes mit zunächst an im Verhältnis zum endogenen Rhyth-

mus falschen Zeitpunkten gegebenen Insulindosen sowie mit falscher KostEinstellung:

Fall 38. Folke R., geb. 1916.

Grossmutter väterlicherseits hat Altersdiabetes.

Pat. war früher stets gesund, erkrankte Sept. 1928 unter zunehmendem Durst und nahm in 3 Wochen 5 kg ab. Im Oktober d. J. wurde der Diabetes festgestellt. 1 Mon. stationäre Behandlung mit strenger Fett-Gemüsediat + 60 g Brot. Der Durst verschwand und das Gewicht stieg. Pat. war müde und hatte schlechten Appetit. Dez. 1929 wiederum Durst, noch geringere Esslust und Atemnot. Pat. nahm in 2 Mon. 10 kg ab, trotz genauer Diät. Febr. 1930 Verschlimmerung, von neuem stationäre Behandlung, Dauer 1 Mon., wobei Insulin eingesetzt wurde, ausserdem strenge Diät. Bei der Entlassung Insulin 32 + 28 I.E. um 8 bzw. 19 Uhr. Besserung,

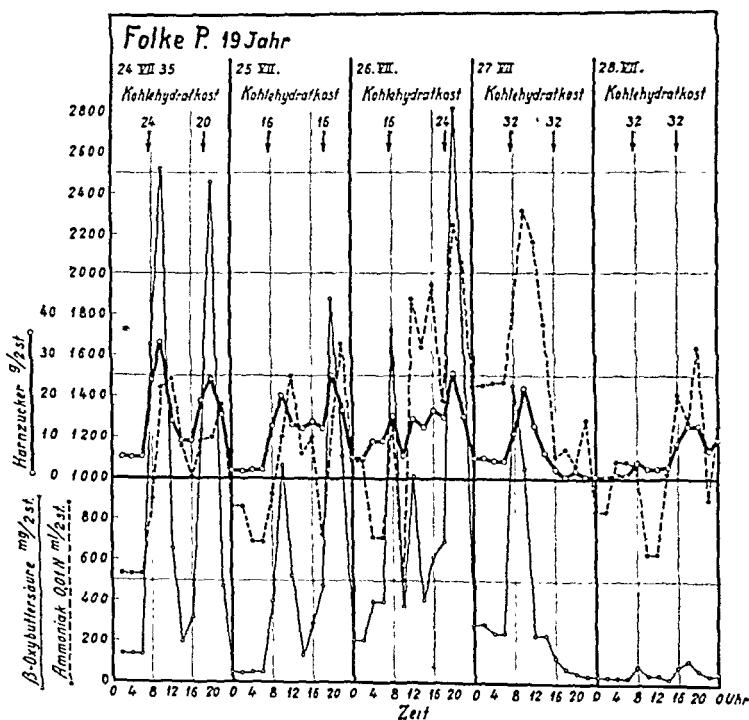


Abb. 94. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes mit anfangs im Verhältnis zum endogenen Rhythmus an falschen Zeitpunkten gegebenen Insulindosen. Man beachte die beiden stark hervortretenden  $\beta$ -Oxybuttersäurewellen am ersten Versuchstage (24. VII.) mit zwei Insulindosen. Nach Verlegung der letzten Insulingabe des Tages auf einen anderen Zeitpunkt (zwei Stunden eher) und leichter Steigerung der Insulinmenge verschwand die Ketonurie.



aber zeitweise Insulinbeschwerden (Schweissausbruch, Schwindel, einmal Bewusstlosigkeit).

Beobachtung des diabetischen Zustands im Juli 1935: Gewicht 69 kg, Länge 187 cm. Ausscheidungsdiagramm siehe Abb. 94.

Anfangs machten sich starke endogene Ketosäurewellen bemerkbar, welche verschwanden, nachdem die zweite Insulindosis von 19 auf 16 Uhr verlegt worden war. Der Insulinbedarf entsprach bei einer gemischten freien Kost 40 + 40 I. E.

Bei der Anfrage im Jahre 1939 lief folgende Antwort auf die Frage nach dem Unterschied im Zustande nach Umlegung der Behandlung im Juli 1935 gegenüber vorher ein: »Viel besserer Gesundheitszustand, bessere Laune und sonst eine Veränderung, welche sich in Worten nicht ausdrücken lässt.« In einem beigegeführten Brief heisst es weiter: »Ich muss wirklich um Entschuldigung für die Gleichgültigkeit bitten, die ich an den Tag gelegt habe, indem ich nicht über die weitere Entwicklung meiner Krankheit nach der revolutionierenden Umlegung 1935 berichtete. Jahr nach Jahr ist indessen vergangen, und ich habe so gut wie alles essen können, ohne die Speisen grammweise abzuwiegen, und habe mich während der ganzen Zeit wunderbar wohl gefühlt.«

Bei der Nachuntersuchung im Mai 1941 war der Gesundheitszustand ausgezeichnet, Pat. wog 76 kg, seitens der inneren Organe keine Veränderungen, Blutdruck normal, Urin frei von Zucker, Ketosäuren und Eiweiss, sowie normaler Blutzucker. Niemals Insulinbeschwerden. Die Insulinmenge, welche in allen Jahren nach der Umlegung 1935 40 + 40 I. E. betragen hatte, wurde auf 36 + 36 I. E. herabgesetzt, was dann beibehalten wurde.

Bei jeglicher Insulinbehandlung ist eine Überdosierung zu vermeiden. Sofern ein etwaiger Insulinüberschuss nicht ausgeschieden oder auf andere Weise vom Organismus unschädlich gemacht wird, kann derselbe eine Gegenregulation mit gesteigerter Reizbarkeit des vegetativen Nervensystems auslösen (vgl. Abb. 31 S. 86). Eine falsche Insulindosierung kann dabei nervöse Reizzustände hervorrufen, welche für den Kranken sehr lästig werden können. Der folgende Fall ist ein Beispiel für einen Diabetiker mit starker Insulinüberdosierung:

*Fall 39. Herbert E., geb. 1894.*

Keine erbliche Diabetesbelastung.

August 1932 zunehmender Durst, Müdigkeit und Abmagerung. Der Diabetes wurde da konstatiert und Pat. einen Monat im Krankenhaus mit strenger Diät (Fett-Gemüse + 50 g Brot) ohne Insulin behandelt. Der Durst liess nach, Pat. war aber nach wie vor müde und

nahm in 3 Monaten weitere 10 kg ab. Im Januar 1933 musste eine Insulinbehandlung mit kleinen Dosen eingeleitet werden. 1936 im Januar ein diabetisches Koma bei Gelegenheit einer Racheninfektion, worauf die Insulindosis stark gesteigert wurde (80 + 40 + 80 I. E. um 6 bzw. 12 u. 19 Uhr). Diese Dosen wurden dann bis Juni 1938 beibehalten, nach wie vor bei strenger Diät mit 50 g Brot als Kohlehydrat. In den letzten beiden Jahren oft Insulinschocke, Pat. konnte bei jedem Anfall bis 2–3 Stunden bewusstlos sein, dabei Krämpfe. Bei der Beobachtung im Juni 1938 ergab sich das in der folgenden Abbildung (95) wiedergegebene Ausscheidungsdiagramm.

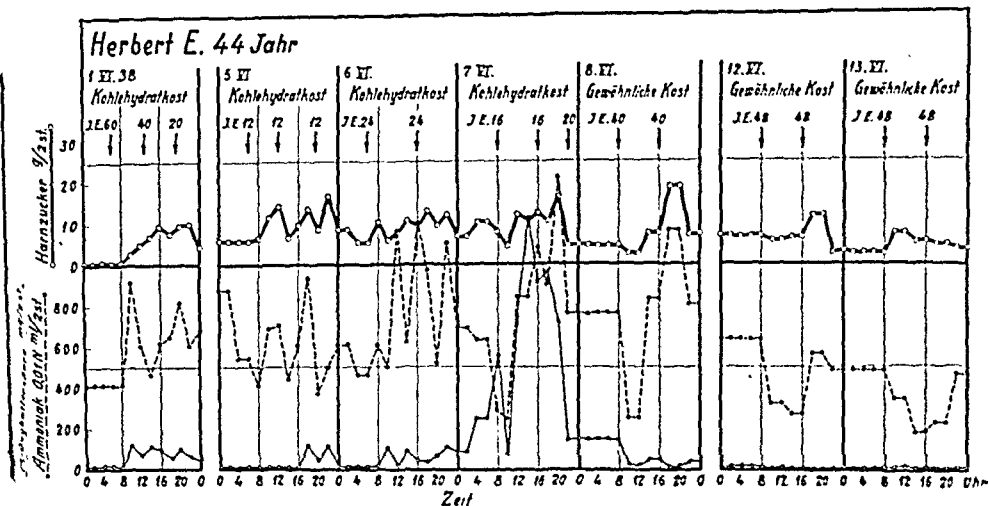


Abb. 95. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes mit zuviel Insulin. Zu beachten sind Ketonurie und mässige Zuckerrdiurese bei 3 Insulindosen, 120 I. E. entsprechend. Bei schrittweiser Verringerung der Insulindosis verschwand die Ketonurie, um wiederzukommen, als die Insulinmenge unzulänglich wurde. Bei zwei dem endogenen Rhythmus angepassten Insulindosen verschwand die Ketonurie. Der Insulinbedarf war 48 + 48 I. E.

Schon am ersten Probetage wurde die Insulinmenge auf 60 + 40 + 20 I. E. herabgesetzt. Bei dieser Insulindosis lag am 1. VI. sowohl Zuckerausscheidung wie Ketonurie vor, was aus dem Diagramm ersichtlich wird. Bei gleichbleibender Kost und Schritt für Schritt verminderter Insulinmenge verschwand während der nächsten Tage die Ketonkörperausscheidung ohne nennenswertes Steigen der Zuckerrdiurese. Als die Insulinmengen am 5. VI. bei 12 + 12 + 12 I. E. angelangt waren, setzte die Ketonurie wieder ein und nahm am 7. VI. stark zu, weshalb eine Extrainsulindosis von 20 I. E. am Abend gegeben werden musste. Der Tagesbedarf an Insulin entsprach 48 + 48 I. E., dem endogenen Rhythmus angepasst. Dabei verschwand jegliche Ketonkörperausscheidung, und Pat. war dann bei einer normalen freien gemischten Kost beschwerdefrei.

Bei diesem letzten Fall hatte die starke Insulinüberdosierung eine Gegenregulation ausgelöst, welche im Verein mit dem Kohlehydratmangel in der Nahrung eine insulinresistente Ketonurie verursacht hatte. Bei verminderter Insulindosis und reichlicher Kohlenhydratzufuhr verschwand diese Ketonurie zeitweise, am wiederzukommen, wenn der wirkliche Insulinbedarf unterschritten wurde. Wurde dann das Insulin dem endogenen Rhythmus richtig angepasst, so hörte die Ketonurie endgültig auf.

Die endogenen  $\beta$ -Oxybuttersäurewellen treten bei verschiedenen Fällen an Hungertagen nicht mit derselben Deutlichkeit hervor.

Bisweilen macht sich im Hunger nur eine starke  $\beta$ -Oxybuttersäureausscheidungswelle bemerkbar (Abb. 96 u. 97), in anderen Fällen wieder finden wir zwei deutliche solche im Laufe der 24 Stundenperiode, eine am Morgen und eine am Nachmittag, mit einem Minimum etwa um 13—15 Uhr (Abb. 98 Fall 42).

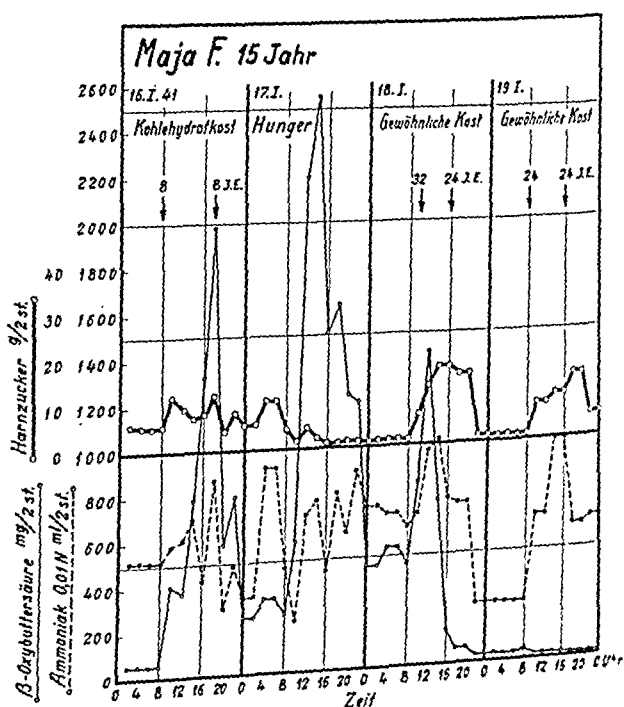


Abb. 96. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes mit einer mark hervortretenden  $\beta$ -Oxybuttersäureausscheidungswelle und nur angedeuteter zweiter Welle während eines Hungertages.

Fall 40. Maja F., geb. 1826.

Keine erbliche Veranlagung für Diabetes nachweisbar. Früher stets gesund gewesen. Im September 1939 zunehmendes Durstgefühl und beginnende Abmagerung. Geringfügige Müdigkeit. Anfang Oktober d. J. wurde der Diabetes festgestellt und die Kranke deswegen 2 Monate lang in einem Krankenhaus behandelt (strenge Fett-Gemüse Kost mit 70 g Brot ohne Insulin). Im Februar 1940 Verschlimmerung, wiederum 1 Monat stationäre Behandlung; nun wurde eine Insulintherapie bei nach wie vor strenger Diät eingeleitet. Bei der Beobachtung im Januar 1941 erhielt man das nebenstehende Ausscheidungsdiagramm 96. Der Insulinbedarf war  $32 + 32$  I. E. bei freier gemischter Kost.

Fall 41. Elsie N., geb. 1892.

Keine erbliche Veranlagung für Diabetes. Im Juli 1933 Paratyphus mit dreiwöchigen Fieber. Im August d. J. wurde Achylie konstatiert. Im April 1934 Iktus. Im September d. J. zunehmendes Durstgefühl und Abmagerung. Damals wurde der Diabetes festgestellt und die Insulinbehandlung eingeleitet, welche dann mit wechselnden Mengen fortbesetzt wurde. 11. I. 1935 Gewicht 59,1 kg, Länge 168 cm. Blutdruck 150/80. Der Diabetestypus wird aus Abb. 97 ersichtlich.

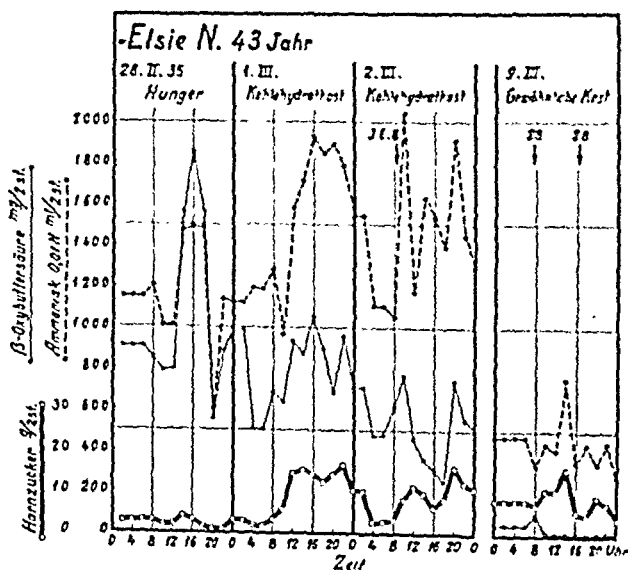
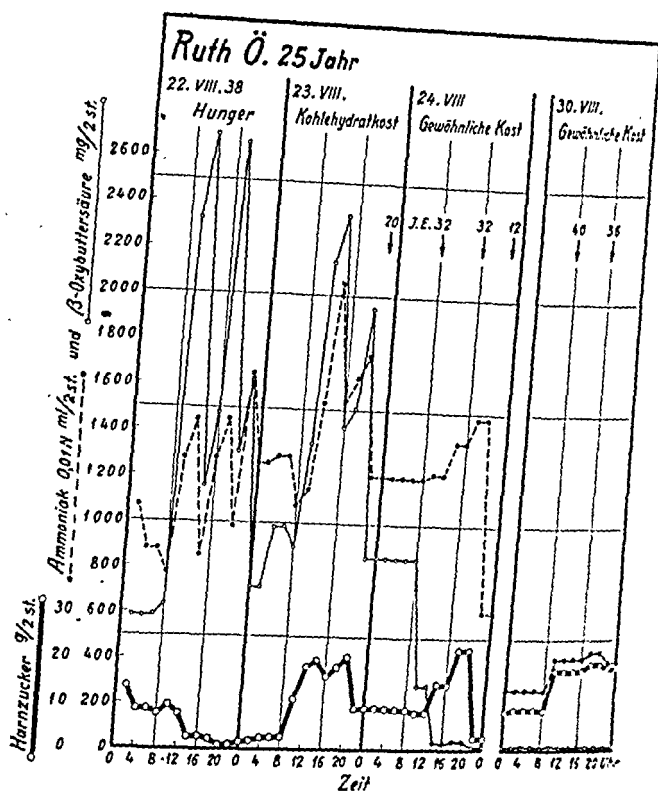


Abb. 97. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes mit nur einer  $\beta$ -Oxybuttersäurewelle am Hungertag, aber mit einer nächtlichen Ausscheidungsquelle.

Fall 42. Ruth Ö., geb. 1913.

Keine Erbanlage für Diabetes.

Im Oktober 1935 zunehmendes Durstgefühl, starke Abmagerung. Der Diabetes wurde im Dezember d. J. konstatiert. Vierwöchige Krankenhausbehandlung mit strenger Diät ohne Insulin. Der Durst verschwand, und die Patientin nahm etwas zu. Bis Herbst 1936 fühlte sie sich ziemlich wohl, dann im Anschluss an eine Halsinfektion zunehmende Verschlimmerung mit stärkerem Durstgefühl, Abmagerung und Azidose. Die Kranke musste sich nach 3 Monaten im Februar 1937 in stationäre Behandlung begeben, bei welcher eine energische Insulinbehandlung (56 + 24 + 56 I.E. pro die) eingeleitet wurde; die Tagesdosis musste wegen Insulinbeschwerden nach und nach herabgesetzt werden. Bei der Beobachtung im August 1938: Länge 170 cm, Gewicht 60,6 kg. Blutdruck 115/70. Der Diabetestypus geht aus Abb. 98 hervor.



b. 98. Ausscheidungsdiagramm bei Diabetes mit zwei stark hervortretenden  $\beta$ -Oxybuttersäureausscheidungswellen während eines Hungertages

Wird die Insulindosis gegeben, wenn die  $\beta$ -Oxybuttersäure zu steigen beginnt, dann geht die Ketonkörperausscheidung sehr rasch zurück, und verschwindet (vgl. S. 296). Dage-

gen bleibt die Zuckerdiurese oft längere oder kürzere Zeit unverändert oder kann sogar eine vorübergehende starke Steigerung aufweisen (Abb. 87).

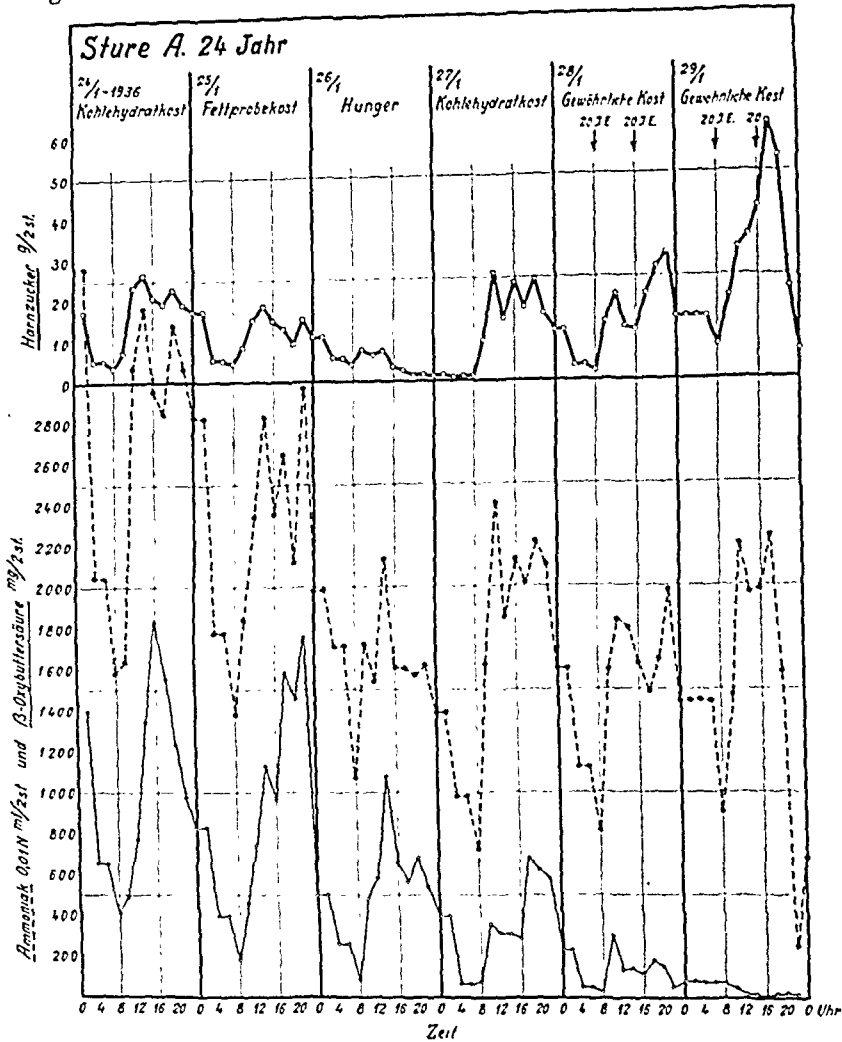


Abb. 99. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes. Man beachte die stark gesteigerte Zuckerdiurese nach Einleitung der Insulinbehandlung zugleich mit dem Verschwinden der Ketonurie (vgl. Abb. 67, S. 296, welche Gesamtstickstoff- und Phosphorausscheidung bei demselben Patienten an den gleichen Tagen zeigt).

Fall 43. Sture A., geb. 1912.

Keine Erbanlage für Diabetes feststellbar.

Im Januar 1935 plötzliches Durstgefühl; trank 12–15 l pro Tag

und nahm in 1 Monat 10 kg ab. Es wurde Diabetes mit Säuren und Eiweiss konstatiert. Krankenhausbehandlung mit strenger Diät ohne Insulin. Zunehmende Verschlimmerung mit Müdigkeit, Durst und herabgesetzter Arbeitsfähigkeit. Pat. konnte im Januar 1936 seinen Beruf infolge von Müdigkeit nicht ausüben. Bei der da vorgenommen Beobachtung bestand ein stark ketosäurenbildender Diabetes, mit schlechter Ammoniakproduktion und absoluter Insulinindikation (Abb. 99 u. 67 S. 396). Als das Insulin eingesetzt wurde, kam es temporär zu einer starken Steigerung der Zuckerdiurese. Dies kann womöglich mit einer vermehrten Zuckerbildung aus Fett durch die Wirkung des Insulins zusammenhängen.

Ist die Insulinmenge im weiteren Verlauf genügend, so geht mit steigendem Körpergewicht auch die Zuckerausscheidung allmählich zurück oder verschwindet, wobei der Insulinbedarf geringer wird. Möglicherweise ist das der Ausdruck einer sekundären Insulinwirkung, mit fortschreitender Verbesserung der Glykogenbildungsfähigkeit des Organismus, nachdem eine Störung im Zwischenstoffwechsel durch die Wirkung des Insulins kompensiert worden war.

*Fall. 44. Evert F., geb. 1901.*

Keine Erbanlage für Diabetes.

Im Herbst 1932 zunehmendes Schwächegefühl, Durst und Abmagerung. Der Diabetes wurde im Februar 1933 konstatiert. Dreiwöchige Krankenhausbehandlung mit strenger Diät (40 g Brot + Fett-Gemüsekost). Insulin wurde kurze Zeit zu Beginn des Krankenhausaufenthalts gegeben, bei der Entlassung aber unter Beibehaltung der strengen Diät ausgesetzt. Im Oktober 1935 Verschlechterung des Allgemeinzustands und stärkeres Durstgefühl, aufs neue Insulinbehandlung, 16 I.E. um  $\frac{1}{2}$  9 Uhr. Der Durst verschwand, und Pat. fühlte sich bis zum Herbst 1936 ziemlich wohl. Da wieder gesteigerter Durst, Wadenkrämpfe und Gewichssturz (8 kg. in 6 Mon.). Beobachtung im Februar 1937: Länge 177 cm, Gewicht 73,3 kg. Blutdruck 170/100, Pulsfrequenz 100. Bei der ersten Untersuchung Eiweiss im Urin, im Sediment hyaline Zylinder sowie rote und weisse Blutkörperchen; nach einiger Zeit wurde der Harnbefund normal, und gleichzeitig sank der Blutdruck auf 125/60. Der Diabetestypus wird aus dem Ausscheidungsdiagramm (Abb. 100) ersichtlich.

Fehlt bei einem Diabetes die  $\beta$ -Oxybuttersäureausscheidung, dann kann man sich bei der Insulinbehandlung in gewissem Grade an die Zuckerdiurese halten. Hier ist jedoch die Sach-





richten kann, wie nach der eindeutigeren  $\beta$ -Oxybuttersäureausscheidung.

Unter der Voraussetzung, dass der Zuckerausscheidung eine mangelhafte Insulinwirkung im Organismus zugrunde liegt, entspricht das periodische Steigen der ausgeschiedenen Zuckermengen im Laufe von 24 Stunden der dissimilatorischen Phase der rhythmischen Lebertätigkeit. Da geben auch die Zuckerausscheidungsdiagramme wichtige Fingerzeige für die Insulinbehandlung. Das Insulin ist in diesem Falle etwa vier Stunden vor der maximalen Zuckerausscheidung zu geben. Fällt die Insulinwirkung auf einen anderen Zeitpunkt, so wird der Effekt geringer (vgl. Abb. 32 S. 87).

Die Erhöhung des Blutzuckergehalts hat bei diesen Fällen wahrscheinlich den Charakter einer Kompensationshyperglykämie, welche die Ketonkörperbildung im Schach hält. Die Hyperglykämie wird in diesem Falle zu einem Ausdruck einer latenten Ketonkörperbildung und in gewissem Sinne zu einem Äquivalent der Ketonurie. Dabei wird sowohl die Hyperglykämie als auch die Zuckerdiurese von Insulin günstig beeinflusst.

Bei den folgenden zwei Fällen finden wir eine ähnliche Zuckerausscheidung im Hunger, aber bei dem einen besteht eine Ketonkörperausscheidung, bei dem anderen dagegen eine Kompensationshyperglykämie ohne Ketonurie:

*Fall 45.* Fingal J., geb. 1906.

Eine Schwester des Vaters leidet an Diabetes.

Pat. bekam im Sommer 1926 starkes Durstgefühl und nahm in 2 Monaten 10 kg ab. Im August d. J. wurde der Diabetes konstatiert, Pat. kam da im Praekoma ins Krankenhaus. Nach 6 Wochen mit ziemlich uneingeschränkter Kost und ohne Insulin entlassen. Fühlte sich 3 Jahre lang wohl. Dann wieder Verschlimmerung, wurde stationär behandelt und mit 24 + 24 I. E. entlassen; diese Dosis wurde in der folgenden Zeit beibehalten. Bei näherer Analyse des Diabetes im Januar 1937 ergab sich das in Abb. 101 a wiedergegebene Ausscheidungsdiagramm.

*Fall 46.* Georg S., geb. 1901.

Ein Bruder des Vaters starb an Diabetes.

Pat. erkrankte im Frühjahr 1923 unter starkem Durstgefühl und nahm in 1 Monat 20 kg ab. Es wurde damals ein Diabetes

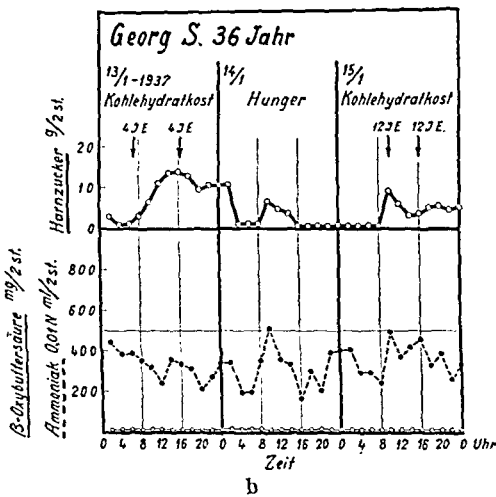
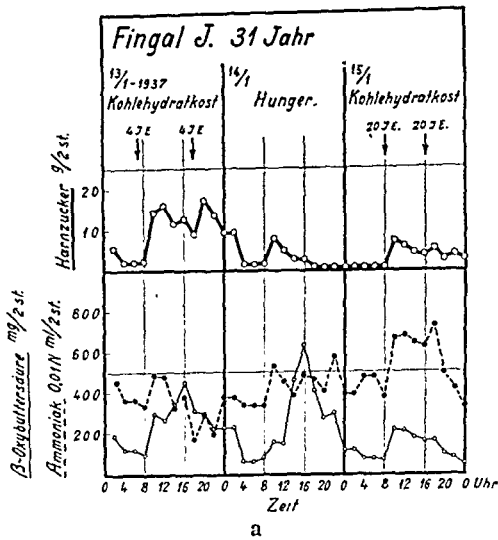


Abb. 101. Ausscheidungsdiagramme mit und ohne Kompensationshyperglykämie im Hunger. a starke Ketonurie, b ohne Ketonurie, mit Hyperglykämie.

Blutzuckerwerte nach Hagedorn am Hungertag 14.I.

	a	b
8 Uhr .....	198 mg %	295 mg %
12 » .....	161 »	223 »
14 » .....	152 »	238 »
16 » .....	150 »	211 »
18 » .....	174 »	230 »
20 » .....	117 »	195 »

ohne Säuren festgestellt. Pat. hielt strenge Diät und fühlte sich bis Dezember 1926 ziemlich wohl. Da rasche Verschlimmerung mit starker Ketosäurenbildung, Pat. wurde im Koma in das Krankenhaus aufgenommen. Er wurde nach 1 Monat mit 12 + 16 I.E. Insulin entlassen, die Dosis wechselte dann, bis 40 + 40 I.E. pro Tag. Bei der Beobachtung im Januar 1937 erhielt man das in Abb. 101 b dargestellte Ausscheidungsdiagramm.

Bei diesen beiden Fällen war die Zuckerausscheidung auffallend gleich. Bei dem ersten wies der Patient am Hungertage nur leichte Hyperglykämie, aber eine starke  $\beta$ -Oxybuttersäureausscheidung auf. Bei dem zweiten hingegen fehlte die Ketonkörperausscheidung, obgleich der Kranke früher eine deutliche Azidose mit Koma gehabt hatte. Hier lag statt dessen eine Kompensationshyperglykämie vor, mit höheren Blutzuckerwerten am Hungertage, welche zwischen 200 und 300 mg% schwankten. Der Allgemeinzustand war bei beiden Patienten während des Hungertages sehr verschieden. Der erste Kranke mit Ketonurie und schlechter Ammoniakausscheidung klagte über Müdigkeit, Kopfschmerzen, Appetitlosigkeit und zeitweise im Laufe des Tages über Übelkeit mit Brechreiz. Der letztere Patient mit Kompensationshyperglykämie vertrug die Nahrungsentziehung sehr gut und hatte während des Tages nur normalen Hunger und Durst. *Beide Fälle wurden von Insulinbehandlung günstig beeinflusst.*

Wenn Hyperglykämie und Zuckerdiurese nicht von Insulinmangel, sondern durch eine Überreizung des blutzuckersteigernden sympathico-adrenalen Systems verursacht sind, dann ist die Insulinbehandlung wirkungslos (vgl. Fall 27 S. 335).

Beim Diabetes vom Typus B ohne Ketonurie sind daher grundsätzlich zwei verschiedene Arten zu unterscheiden:

1. Der Regulationsmechanismus des Blutzuckers kann bei einem Teil der Fälle hinsichtlich seiner Wirkung ziemlich ungestört sein. Infolgedessen kann eine kompensatorische Hyperglykämie eine vorhandene Stoffwechselstörung bessern und die Entstehung einer Ketonurie verhindern. Ein B-Diabetes von dieser Art ist eigentlich ein latenter A-Diabetes. Derselbe kann bei ungenügender Kompensationswirkung der Blutzuckerregulation leicht in einen ketonkörperbildenden Diabetes umschla-

gen, wodurch eine erheblich energischere Insulinbehandlung erforderlich werden kann (vgl. oben Fall 46).

2. Bei anderen Fällen von B-Diabetes ist dagegen eine Überreizung des blutzuckersteigernden Regulationssystems die Ursache des hohen Blutzuckerspiegels. Hier haben wir die primäre Störung nicht in einem veränderten chemischen Umsetzungsprozess sondern in einer Gleichgewichtsverschiebung der neurohormonalen Blutzuckerregulation zu suchen. Diese Diabetesformen sind daher letzten Endes auf eine vegetativ-nervöse Störung funktioneller oder organischer Art zurückzuführen. Sie sind in grossem Umfang insulinresistent, da die Störung des Kohlehydratstoffwechsels bei diesen Fällen nicht auf einem Insulinmangel beruht. In vielen Fällen ist hier eine kausale Therapie sehr schwer, und die einzige Behandlung, welche möglich ist, besteht darin, dass man die Glykogenbildung des Organismus erleichtert und den Regulationsmechanismus durch passende Nahrungsbeschränkung vor Überbürdung zu schützen sucht (vgl. III. Abschnitt). Wird bei diesen Fällen Insulin gegeben, so kann eine gesteigerte Gegenregulation in einem von vornherein schon überreizten vegetativen Nervensystem die Folge sein, und es kann zu Komplikationen verschiedener Art kommen. Ein Beispiel für einen B-Diabetes dieser Art ist Fall 27 S. 335.

Bei einem Diabetes kann es sich wie bei einem chronischen Fieber um einen in gewissen Grenzen stabilen, in anderen Fällen aber auch um einen höchst veränderlichen Zustand handeln, mit grosser Labilität und beträchtlichen Schwankungen des Insulinbedarfs. Dies gilt namentlich für gewisse Formen von Regulationsdiabetes. Dabei können durch gelegentliche Störungen im regulatorischen System plötzliche Verschlimmerungen eintreten. Es kann da rasch ein erheblicher Insulinmangel mit einer Ketonkörperbildung zustande kommen, welcher eine wirksamere Insulinbehandlung mit grösseren Dosen erforderlich macht.

Die auslösenden Faktoren für die Regulationsstörung sind mannigfach. Ein Trauma, ein psychischer Schock, eine Gehirnreizung, ein epileptischer Anfall, eine Überanstrengung, Schlafmangel, ein Schmerzzustand, eine Infektion u. a. m., alle

diese Momente können eine blitzartige Verschlimmerung eines bestehenden Diabetes durch eine reflektorische Rückwirkung auf die regulatorischen Zentren bewirken. Bei derartigen Prozessen entwickelt sich die Ketonkörperbildung nicht selten äusserst rasch und kann zum Koma führen, wenn nicht eine Insulinbehandlung unverzüglich eingeleitet oder bei im Gange befindlicher solcher die Dosis erheblich gesteigert wird, evtl. mit temporärer Verabfolgung zusätzlicher Insulingaben.

Wenn der Organismus nach einer gelegentlichen Störung wieder in das Stoffwechselgleichgewicht gelangt ist, kann die Insulinmenge herabgesetzt werden. Die Dosis soll da vermindert und nicht in unnötiger Höhe beibehalten werden.

*Das führende Prinzip bei jeglicher Diabetesbehandlung muss darin liegen, dass einem vorhandenen Biokatalysatormangel abgeholfen und eine Verschiebung des Regulationsgleichgewichts nach Möglichkeit ausgeglichen wird.* Bei Insulinmangel ist dieser in erster Linie zu kompensieren. Handelt es sich um einen bedrohlichen Diabetes mit starker Ketonkörperbildung und ungenügender Ammoniakproduktion, dann ist die Insulinbehandlung vital indiziert. Bei Diabetesformen vom Typus A kann die Indikation der Insulinbehandlung eine relative oder absolute sein und von Ketonkörperausscheidung, Ammoniakbildung und Verhalten des Körpergewichts abhängen. Das gleiche gilt für kompensationshyperglykämische Diabetesformen vom Typus B. Bei jeder ketonkörperbildenden Diabetesform ist in der Regel Insulin in höherem oder geringerem Grade indiziert. Bei anderen Fällen von B-Diabetes, wo es sich nicht um Insulinmangel handelt, können es insulinresistente Formen sein, bei welchen die Insulinbehandlung sogar kontraindiziert sein kann (vgl. Fall 27 S. 335).

*Die Insulinbehandlung muss nach den oben angegebenen Grundsätzen der endogenen Stoffwechselperiodizität angepasst werden.* Dabei muss das Insulin in richtiger Menge und rechtzeitig gegeben werden. Für den Zeitpunkt ist nicht die Nahrungsaufnahme ausschlaggebend, sondern die neurohormonale endogene Regulationsperiode (vgl. S. 87). Nur bei Beachtung dieser Gesichtspunkte ist es möglich, die harmonische Interferenz zwischen Insulinbehandlung und regulierenden Kräf-

ten des Stoffwechsels zustande zu bringen, welche sich für den Patienten in einem gesteigerten Wohlbefinden und der Abwesenheit jeglicher Insulinbeschwerden äussert.

Auch ohne die Mitwirkung des Insulins können bei Störungen im basalen Regulationssystem des Blutzuckers im Hunger hypoglykämische Zustände eintreten. Selbstverständlich werden diese gesteigert, wenn da noch Insulin zugeführt wird. Aus diesem Grunde ist es wichtig, dass ein Insulinpatient mit einer derartigen Störung nicht Hunger leidet, und dass nicht zu lange Zeit zwischen Insulindosis und Mahlzeit vergeht. Sonst droht die Gefahr der Insulinbeschwerden und hypoglykämischen Reaktionen. Namentlich bei diesen Fällen ist es notwendig, dass die Insulinbehandlung dem endogenen Rhythmus nach Möglichkeit angepasst wird, um eine unnötige Gegenregulation und abnorme Reizzustände im neurohormonalen Regulationsapparat zu verhindern.

*Eine Insulintherapie, welche auf die endogenen periodischen Schwankungen im Zwischenstoffwechsel keine Rücksicht nimmt, baut auf physiologisch unrichtigem Grunde.* Während der Phase der rhythmischen Lebertätigkeit, in der der Körper die Hilfe des Insulins braucht, soll letzteres mit abgepassster Stärke wirken. Andererseits ist der Organismus vor der unnötigen Insulinwirkung zu schützen, wenn diese nicht erforderlich ist. Dies ist auch bei Anwendung von Insulin mit verzögerter Wirkung zu beachten.

## Depotinsulin

Die direkt blutzuckersenkende Wirkung des Insulins hält ca. 4 Stunden an (vgl. Abb. 30 S. 86). In der Absicht, diese Wirkungszeit zu verlängern, hat man auf mehr oder weniger unklarer theoretischer Grundlage Versuche angestellt, zu einem Depotinsulin mit protrahierter blutzuckersenkender Wirkung zu gelangen. Dabei wurde das Insulin mit verschiedenartigen kolloidalen Substanzen mit vermutlich resorptionsverzögernder Wirkung gemischt, z. B. Gummiarabikum, Gallensäuren,

Olivöl, Lezithin, Cholesterinemulsion usw., evtl. mit Bildung komplexer Additionsverbindungen.

In neuerer Zeit hat ein Niederschlag des Insulins mit einem stark basischen Eiweisskörper nach *Hagedorn* in Form von Protamininsulin, welches bei pH 7,8 fast wasserunlöslich ist, bei der Insulinbehandlung ausgedehnte Verwendung gefunden. Die Voraussetzung der Insulinwirkung ist wahrscheinlich eine Auflösung etwaiger Peptid- oder anderer Bindungen in der unlöslichen Eiweissverbindung. Die Bedeutung der dabei frei werdenden Eiweisskomponente ist nicht bekannt. Möglicherweise kann dieselbe irgendwelche Immunisierungsprozesse im Organismus auslösen, eine Proteintherapie nach *Singer*, was zu einer therapeutischen Wirkung beitragen kann.

Abgesehen von der direkt blutzuckersenkenden Wirkung erstrecken sich die indirekten Effekte des Insulins über längere Zeit, wenn dasselbe am rechten Zeitpunkt mit Bezug auf den endogenen Stoffwechselrhythmus gegeben wird (vgl. Abb. 32 S. 87). Man kann sich daher die Frage vorlegen, ob die sog. protrahierte Wirkung des Depotinsulins nicht vielleicht nur eine scheinbare ist und darauf beruht, dass die Wirkung des

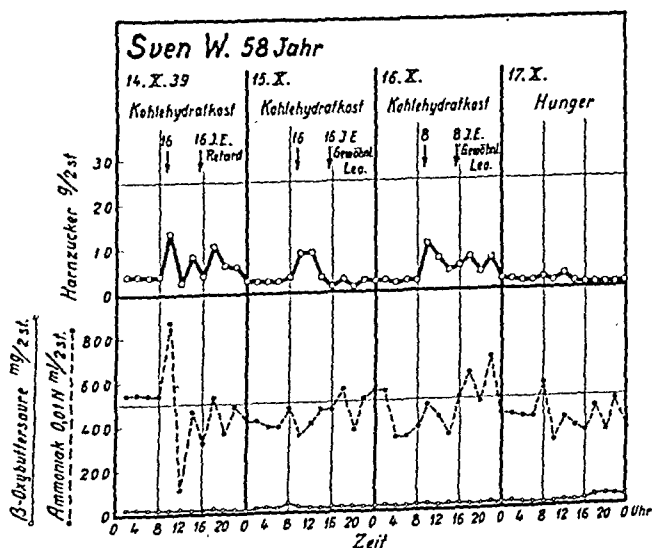


Abb. 102. Ausscheidungsdiagramm bei mit Insulin behandeltem Diabetes ohne Ketonkörperbildung, wo Insulin Retard bei bleichbleibender Kost durch gewöhnliches Insulin ersetzt wurde. Zu beachten ist, dass Insulin Retard hinsichtlich der Zuckerdiurese keine bessere Wirkung hat.

Insulins in eine geeignetere Phase der periodischen Lebertätigkeit fällt. In vielen Fällen tritt nämlich bei gleichbleibender Kost kein deutlicher Unterschied in den Ausscheidungsdiagrammen zutage, wenn das Protamininsulin gegen gewöhnliches Insulin ausgewechselt wird (Abb. 102).

Einen anderen Weg hat *Clausen* beschritten, um eine verzögerte Resorption des Insulins zu erzielen. Dadurch, dass das Insulin mit Adrenalin gemischt wird, soll bei Injektion desselben eine periphere Gefäßkontraktion mit Erschwerung der Resorption stattfinden, in Analogie mit der Wirkung des Adrenalins bei der Lokalanästhesie. Es ist zweifelhaft, ob der örtlich gefäßverengernden Wirkung des Adrenalins bei dem Bestreben, den Insulineffekt zu verlängern, eine nennenswerte Bedeutung zukommt, da sich die Wirkung des Insulins ja nicht wie die der Lokalbetäubungsmittel auf die engste Umgebung der Injektionsstelle beschränkt, sondern generell in den Stoffwechsel, wahrscheinlich primär in den Lipoidumsatz, eingreift. Ausserdem ist das Adrenalin im glykoregulatorischen System ein Insulinantagonist, der überdies die Umsetzung des Glykogens beeinflusst (s. S. 45 u. 88).

Bei Diabetesformen vom Regulationstypus mit Übererregbarkeit des sympathico-adrenalen Systems erscheint die Kombination Adrenalin-Insulin besonders bedenklich. Das Adrenalin kann hier Herzbeschwerden und Blutdrucksteigerungen auslösen, welche zur Vorsicht bei allgemeiner Anwendung des Adrenalin-Insulins mahnen, insbesondere bei einer den diabetischen Zustand begleitenden Hyperthyreose infolge gesteigerter thyreotroper Hypophysenwirkung.

Bei Diabetesformen dagegen mit herabgesetzter Nebennierenfunktion und Ausfall von kontrainsulären Reizimpulsen mit dadurch verursachter Neigung zu spontaner Hypoglykämie kann wahrscheinlich die Kombination Adrenalin-Insulin dem reinen Insulin vorzuziehen sein. Die günstigere Wirkung beruht dabei nicht auf einer verzögerten Resorption des Insulins, sondern auf dem Eingreifen des Adrenalins in den Stoffwechselprozess.

Die Steigerung der Glykogenbildung in der Leber durch das Adrenalin (vgl. S. 43) ist dabei ein für den Kohlehydrat-



Eine verzögerte und verstärkte Insulinwirkung lässt sich bisweilen nachweisen, wenn gleichzeitig mit dem Insulin Zinksalze zugeführt werden (Scott und Fisher, Carlborg u. a.). Die Wirkung ist nicht konstant (Abb. 103).

Es ist möglich, dass die Zinkionen bei den intermediären Stoffwechselprozessen irgendwie die Rolle eines Katalysators spielen, und dass es daher bei Zinkmangel im Organismus eine durchaus angebrachte Therapie sein kann, wenn man versucht, durch passende Zufuhr von Zink diesen Zinkmangel zugleich

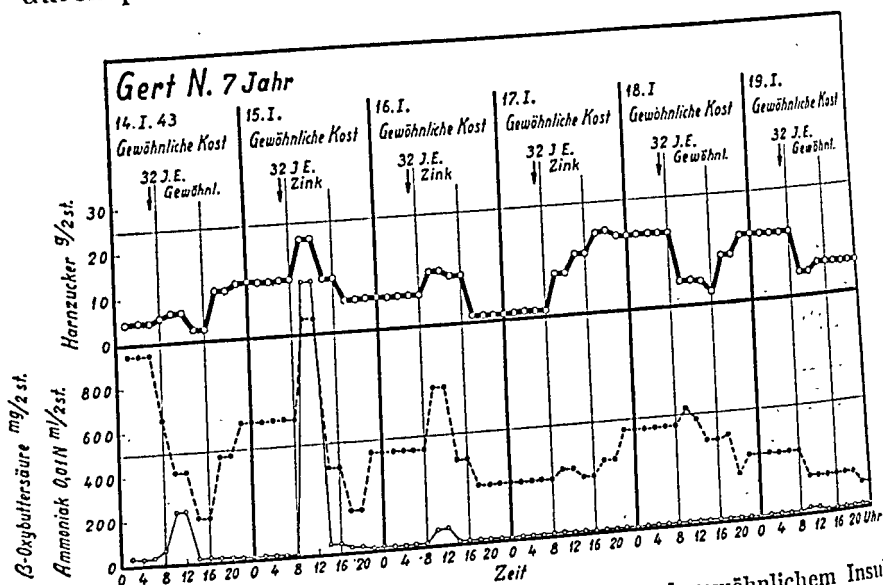


Abb. 103. Vergleichende Versuche mit Zinkinsulin und gewöhnlichem Insulin. Die Verzögerung der Insulinwirkung ist nicht konstant (vgl. Harnzucker-  
ausscheidung am 16. I. und 17. I.). Bei diesem Diabetesfall ist keine sichere  
Verstärkung der Insulinwirkung durch Zink zu konstatieren. Die Ausschei-  
dung variiert gemäss dem endogenen Rhythmus.

mit einer sonst lege artis durchgeführten Insulinbehandlung vorsichtig zu kompensieren.

Zinkionen scheinen als katalytisch wirkende Faktoren bei enzymatischen Prozessen auf verschiedene Weise in den Kohlehydratstoffwechsel hemmend oder förderlich eingreifen zu können (vgl. S. 123, 127 u. 254). Im Überschuss können sie toxisch wirken und dauernde Störungen im Kohlehydrataushalt des Organismus hervorrufen. Der beste Beweis hierfür ist, dass sich ein akuter Diabetes in direktem Anschluss an eine Zinkvergiftung entwickeln kann (vgl. Fall 5 S. 266). Dieser Sachverhalt mahnt auch zur Vorsicht bei der Verwendung des Zinks als Ergänzung der Insulinbehandlung.

Auf der anderen Seite spielt das Zink offenbar eine biologisch wichtige Rolle. *Scott* und *Fisher* (1934) fanden, dass die Gegenwart kleiner Mengen von Zinksalzen erforderlich war, um kristallinisches Insulin zu erhalten, und dass die Anwesenheit des Zinks die Wirkung des Insulins verlängerte. Diese Autoren stellten in dem kristallinischen Insulin 0,52 % Zink fest, welches im Insulinmolekül so fest gebunden war, dass es sich durch wiederholte Umkristallisierungen nicht entfernen liess. Auch durch Kataphorese liess sich nach *Cohn* das Zink nicht von dem Insulin trennen. In gewöhnlichen, nicht kristallinischen Insulinpräparaten haben *Sahyun* und *Feldkamp* sowie *Rabinowitsch* Zink in zwischen 0,01 und 0,3 % der Trockensubstanz des Insulins wechselnder Menge nachgewiesen. Zu gewöhnlichem Insulin muss daher etwas Zink zugesetzt werden, um diejenige Zinkmenge zu erreichen, welche bei der Kristallisation im Insulinmolekül gebunden wird.

Nach *Bertrand* beträgt der Zinkgehalt des menschlichen Körpers etwa die Hälfte des Eisengehalts und entspricht ungefähr 2 mg%; der Zinkgehalt ist also bei einem Erwachsenen von 70 kg ca. 1,4 g Zink. Eine gewisse Zinkmenge ist schon beim Neugeborenen vorhanden. Dies deutet darauf hin, dass das Zink eine funktionelle Aufgabe im Organismus zu erfüllen habe. Nach *Burstein* enthält das menschliche Blut in der Norm etwa 0,52 mg% Zink. In verschiedenen Organen wechselt der Zinkgehalt in hohem Grade; er ist in tätigen sekretorischen Drüsen besonders hoch, in ruhenden Organen, Muskulatur,

Knochen und Fettgewebe geringer. *Simakov* gibt an, dass beim Menschen im Mittel die Hypophyse 112 mg%, der Hoden 12,3 mg%, die Schilddrüse 12,6 mg% und die Bauchspeicheldrüse 1,6—5,6 mg% Zink enthält.

Nach *Simakov* soll der Zinkgehalt der Drüsen normalerweise nicht mit steigendem Lebensalter abnehmen. *Bertrand* und *Benzon* zeigten bei Versuchen an Ratten, dass die Lebensdauer der Tiere ausnahmslos geringer war, wenn dieselben zink-freies Futter erhielten, als wenn ihre Nahrung eine kleine Menge Zink enthielt. Bei Untersuchungen über den Zusammenhang zwischen Vitamin- und Zinkgehalt fanden *Bertrand* und *Bhattacharjee*, dass Mäuse, deren Nahrung Vitamin A, B<sub>1</sub>, B<sub>2</sub> und D reichlich, aber kein Zink enthielt, eine mittlere Lebensdauer von 16,9 Tagen hatten; wurden zu derselben Nahrung kleine Mengen Zinksalze hinzugefügt, so stieg die mittlere Lebensdauer auf 64,4 Tage. Zwischen Zink und Vitaminwirkung scheint demnach ein gewisser Synergismus zu bestehen.

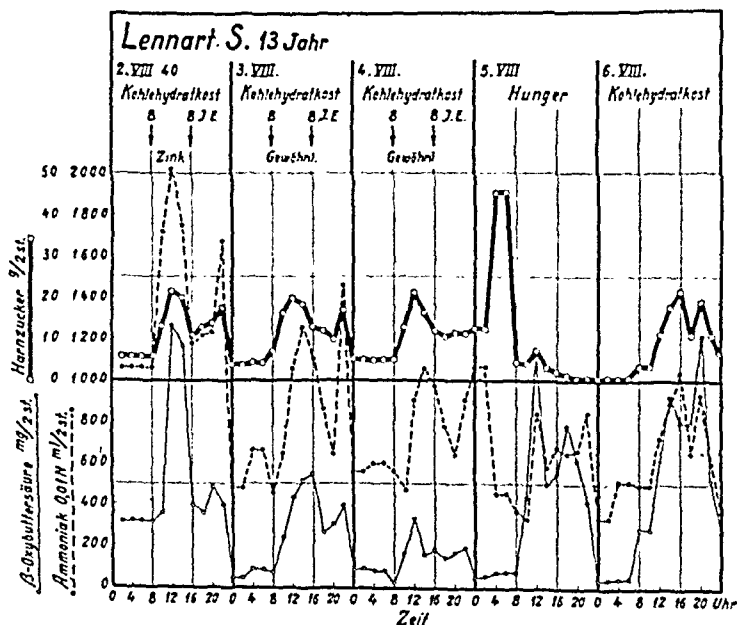
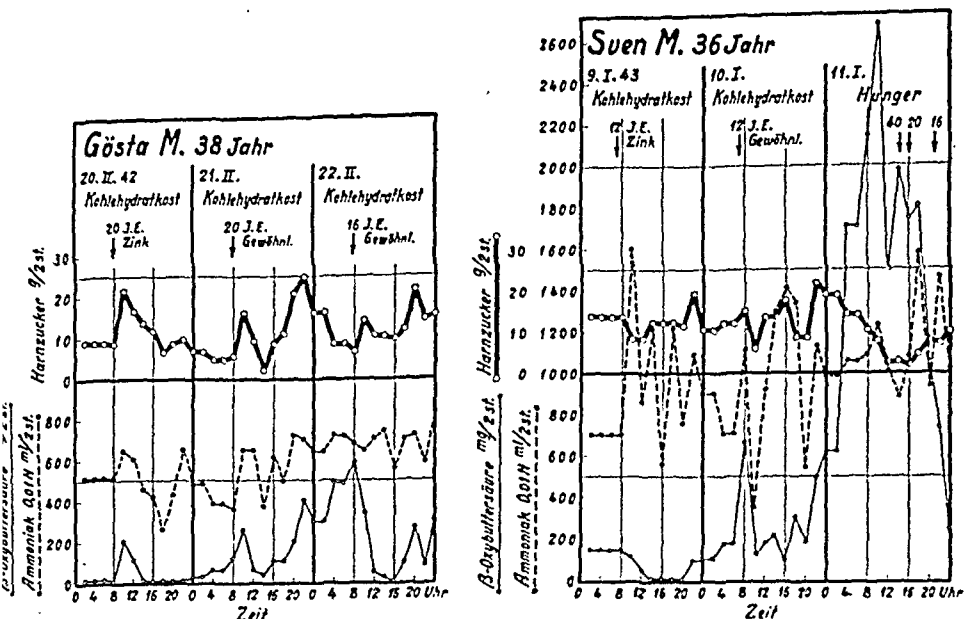
*Scott* und *Fisher* konstatierten eine Herabsetzung des Zink- und Insulingehalt in von Diabetikern stammenden Bauchspeicheldrüsen.

*Horvai* hat festgestellt, dass der Zinkgehalt des Pankreas bei normalen Hunden zwischen 13,4 und 24,9 mg% der Trockensubstanz wechselt, mit einem Mittelwert von 19,5 mg%. Bei hypophysenlosen Hunden schwankte der entsprechende Wert zwischen 14,6 und 35,6 mg% und betrug im Mittel 22,4 mg%. Bei an Kachexie eingegangenen Hunden war der Zinkgehalt auf im Mittel 12,5 mg% gesunken. Im Pankreas der hypophysenlosen Tiere lag gleichzeitig mit der Vermehrung des Zinkgehalts eine histologisch nachweisbare Zunahme der Anzahl von Langerhansschen Inseln vor.

Über die biochemische Wirkung des Zinks vgl. S. 123 u. 127.

Bei gewissen Diabetesfällen hat Zinkinsulin eine bessere Wirkung als gewöhnliches Insulin (Abb. 104).

Vermutlich herrscht bei einem Teil der Diabetesfälle ein Mangel an verfügbaren Zinkionen im Organismus, bei anderen Fällen nicht. Im ersteren Falle wirkt das Zinkinsulin besser, im letzteren besteht kein Unterschied zwischen der Wirkung des Zinkinsulins und des gewöhnlichen Insulins.



Bei anderen Diabetesfällen wirkt dagegen das gewöhnlich Insulin besser als Zinkinsulin (Abb. 105 u. 106).

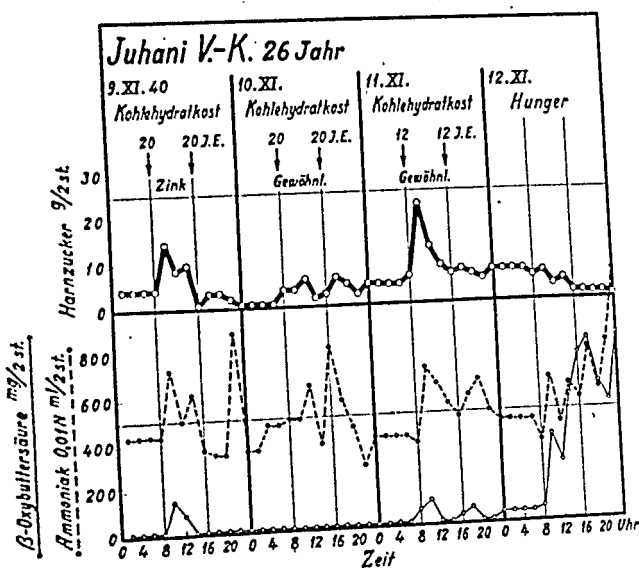


Abb. 106. Ausscheidungsdiagramm bei ketonkörperbildendem Diabetes. Gewöhnliches Insulin wirkt bei diesem Fall besser als Zinkinsulin.

Wahrscheinlich lässt sich dem Zinkmangel im Organismus ebensogut durch perorale Zinkzufuhr abhelfen, was von vielen Gesichtspunkten aus vorzuziehen wäre, aber diese Frage ist nicht hinreichend geklärt. Unter allen Umständen muss auch die Behandlung mit Zinkinsulin dem endogenen Rhythmus angepasst werden.

Aus den obigen Ausführungen geht hervor, dass Zink für die normalen Lebensfunktionen erforderlich zu sein scheint und im Organismus physiologisch vorkommt. Bei Überdosierung kann das Zink jedoch schädliche Wirkungen haben. Die Behandlung mit Zinkinsulin kann daher keine Normalmethode sein. Das Zinkinsulin darf nur bei bestimmten Indikationen angewendet werden, und zwar, wenn im Organismus Mangel an wirksamen Zinkionen herrscht. Ist dies nicht der Fall, dann hat das Zinkinsulin keinen besseren Effekt als gewöhnliches Insulin, kann aber dafür Beschwerden verschiedener Art hervorrufen. Bei einer vergleichenden Untersuchung an 19 Patienten ergab sich, dass 10 Kranke gewöhnliches Insulin besser

vertragen als Zinkinsulin, 4 wurden von Zinkinsulin etwas günstiger beeinflusst und 5 bemerkten keinen Unterschied zwischen der Wirkung der beiden Insulinarten. Selbstverständlich lassen sich lediglich aus dem Verlauf der durch Bestimmung der Reduktionswerte erhaltenen Blutzuckerkurve keine Schlüsse über das Eingreifen des Zinkinsulins in den Stoffwechselprozess ziehen, da das Zink wahrscheinlich auch ganz andere, noch unbekannte Aufgaben im Körper zu erfüllen hat. Bei einigen Fällen verursachte das Zinkinsulin nervöse Nebenerscheinungen, Unruhe, Hautjucken, Herzklopfen und manchmal eine Beeinträchtigung des Allgemeinbefindens, Beschwerden, welche beim Übergang vom Zinkinsulin zu gewöhnlichem Insulin, *lege artis* dosiert, nachliessen oder verschwanden. Leider ist es zur Zeit nicht möglich, objektiv festzustellen, ob Zinkmangel im Organismus vorliegt.

## Psychische Faktoren und Diabetesbehandlung

Eine Voraussetzung für den guten Erfolg der Diabetestherapie ist, dass sich die Patienten im psychischen Gleichgewicht befinden und sich ihrer Krankheit wegen keine unnötigen Sorgen machen. Bei jedem Diabetes sind nervöse Störungsmomente durch die Wirkung der neurohormonalen Blutzuckerregulation in höherem oder geringerem Grade beteiligt. Namentlich beim Regulationsdiabetes kann hierdurch der Kohlenhydratstoffwechsel auch von übergeordneten Gehirnzentren aus durch Impulse psychischer Art beeinflusst werden (vgl. VI. Abschnitt). Auf der anderen Seite muss ein Diabetiker unter der erforderlichen ärztlichen Aufsicht stehen, damit jederzeit ein rasches Eingreifen möglich ist, wenn irgendeine unvorhergesehene Verschlimmerung Platz greifen sollte. Ein Regulationsdiabetes kann äusserst labil sein. Ein plötzlicher Schreck, ein Trauma, eine hinzukommende Infektion oder irgendwelche anderen unberechenbaren Faktoren können bei diesen Fällen eine hochgradige Verschlechterung der Stoffwechsellage auslösen. Dabei kann der Insulinbedarf wesentlich steigen, was

sich u. a. in zunehmender Ketonurie zeigt. Besonders bei diesen Fällen ist es eine *Conditio sine qua non* für eine erfolgreiche Therapie, dass die Insulinbehandlung *lege artis* dem augenblicklichen Insulinbedarf gleitend angepasst wird, so dass die Kranken der ständigen Angst vor üblen Zufällen enthoben sind. Ein wertvolles Hilfsmittel der praktischen Diabetesbehandlung sind hierbei regelmässige Urinuntersuchungen.

## Blutzuckerbestimmung und Urinuntersuchung

Infolge des vieldeutigen Charakters des Blutzuckerwerts (vgl. S. 286) ist eine gewöhnliche Blutzuckerprobe für die Beurteilung des Zustands von untergeordneter Bedeutung und nur dann wertvoll, wenn es sich darum handelt, zu entscheiden, ob der Blutzuckergehalt hoch oder gering ist. Ein klareres Bild gibt dagegen die Urinuntersuchung. Durch diese kann man sich einen bestimmten Begriff von dem Kalorieverlust durch die Zuckerdiurese, von dem Schweregrad der Stoffwechselstörung durch die  $\beta$ -Oxybuttersäureausscheidung und von der etwaigen Komagefahr durch die Ammoniak- $\beta$ -Oxybuttersäurebilanz machen. *Für eine laufende Kontrolle des diabetischen Zustandes ist daher eine systematische Urinuntersuchung von weit grösserem Wert als eine einfache Blutzuckeruntersuchung.* Ist der Diabetestypus bekannt, dann geben die am Tage und in der Nacht ausgeschiedenen Zucker- und Ketosäuremengen bei bekannter Ernährung klaren Aufschluss über die Stoffwechsellage und den Insulinbedarf, können als Richtschnur einer rationellen Diabetesbehandlung dienen und ermöglichen eine gute Kontrolle der Schwankungen des diabetischen Zustands.

## Schlusswort

Bei den Störungen im Zwischenstoffwechsel beim Diabetes scheinen zwei grundlegende Prozesse des biologischen Hexosenabbaus im Mittelpunkt zu stehen: *Phosphorylierungsstörungen*

und Störungen in der oxydativen Abbauphase. Im ersteren Falle kann eine Ketonkörperbildung vorliegen, im letzteren eine Anaerobie. Es ist jedoch noch nicht möglich, diese Störungen in allen Fällen mit Sicherheit zu unterscheiden, es scheint aber, als ob den durch klinische Studien gefundenen Diabetesformen vom A- bzw. B-Typus gewissermassen die Störungen im Phosphorylierungsprozess resp. in der oxydativen Abbauphase der Kohlehydraten entsprechen. Eine genauere Klarstellung der Stoffwechselstörung mit Rücksicht auf veränderte Partialfunktionen in dem biologischen Verlauf ist doch eine der Aufgaben zukünftiger Forschung.

Zur Zeit steht die Diabetestherapie in vielen Beziehungen auf schwankendem Boden. Der reale Wert von therapeutischen Prinzipien und Arbeitshypothesen lässt sich nicht durch theoretische Betrachtungen ergründen. Nur direkte Erfahrungen an einem klinischen Diabetesmaterial, welches früher nach anderen Prinzipien behandelt worden war, wo aber dann die Behandlung im Einklang mit den Postulaten der Arbeitshypothese umgelegt wurde, vermögen zur Beantwortung der Frage zu führen, welche Behandlungsgrundsätze die richtigen sind.

Seit 1933 habe ich meiner Diabetestherapie diejenigen Anschauungen zugrunde gelegt, welche aus der vorliegenden Darstellung ersichtlich werden und etwa 1900 Diabetespatienten eingehender untersucht. Die Grundprinzipien waren dabei folgende:

1. Klarstellung des diabetischen Zustands mit Rücksicht auf den endogenen Rhythmus in der oben angegebenen Weise (vgl. S. 325).
2. Abhilfe für etwaige Mangelzustände, wo dies möglich ist.
3. Anpassung der Insulinbehandlung an den endogenen Stoffwechselrhythmus.
4. Einstellung auf eine normale gemischte, freie Kost bzw. Ernährung mit denjenigen Kost einschränkungen oder Vorschriften, welche bei dem speziellen Diabetesfall auf Grund des Charakters der Krankheit erforderlich sind.

Um zu einer Auffassung über den Gesundheitszustand der Diabetespatienten vor und nach der Umlegung der Behandlung



gemäss diesen Grundsätzen zu gelangen, wurde in den Jahren 1939 und 1940 eine Umfrage unter vorwiegend bedrohlichen Diabetesfällen in die Wege geleitet, bei welchen die Beobachtungszeit lang genug gewesen war, um den Sachverhalt zu beleuchten. Um einen Vergleich zwischen älteren und neueren Behandlungsprinzipien zu ermöglichen, wurden bei der Auswahl dieses klinischen Materials folgende Bedingungen berücksichtigt:

1. Der Patient soll mindestens ein Jahr vor der Umlegung der Behandlung *seines Diabetes wegen* während einer Zeit von *wenigstens einem Monat* und ohne Komplikationen Krankenhausbehandlung erhalten haben.

2. Die Umlegung der Behandlung entsprechend den angegebenen Richtlinien hatte während einer Beobachtungszeit von *höchstens 14 Tagen* zu erfolgen.

3. Nach Umlegung der Behandlung musste mindestens ein Jahr vergangen sein, bevor die Patienten oder Angehörige derselben die Frage nach der Veränderung des Gesundheitszustands nach Umlegung der Behandlung beantworten durften.

Unter den Patienten, deren Diabetesbehandlung am Zeitpunkt der Umfrage den angegebenen Prinzipien gemäss umgelegt worden war, befanden sich 185 Fälle, welche diese Bedingungen erfüllten.

In der folgenden Tabelle, in der die Diabetesfälle nach dem Alter beim Ausbruch der Krankheit geordnet sind, ist das Resultat dieser Umfrage auszugsweise angegeben.

# SCHLUSSTABELLE

	Geburts-jahr	Diabetes konstatiert (Jahr)	Alter beim Ausbruch d. Krankheit (Jahre)	Krankenhausbehandlung wegen Diabetes vor Umlegung d. Behandlung		Insulinbehandlung		Beobachtungszeit für Umlegung d. Behandlung	Typus
				Anzahl Male	Gesamtdauer in Wochen	vor d. Umlegung	seit		
Alice G.	1930	1933	3	7	20	ja	1933	Mai 1938	0
Sigrid W.	1927	1930	3	3	22	ja	1935	Nov. 1937	0
Eva T.	1927	1931	4	2	24	ja	1931	Jan. 1937	0
Gunnel E.	1932	1936	4	1	4	ja	1936	April 1937	A
Sven P.	1928	1932	4	1	5	ja	1932	Jan. 1938	0
Hugo E.	1927	1931	4	1	8	ja	1932	Aug. 1935	A
Hillevi H.	1928	1932	4	6	24	ja	1932	Okt. 1938	0
John S.	1920	1924	4	6	16	ja	1925	Jan. 1938	0
Inga C.	1927	1932	5	2	10	ja	1932	Jan. 1938	0
Nils A.	1930	1935	5	2	6	ja	1935	Dez. 1936	0
Lennart, M.	1932	1937	5	2	10	ja	1937	Okt. 1938	0
Boel E.	1931	1936	5	2	9	ja	1936	Sept. 1938	0
Bengt G.	1923	1929	6	6	14	ja	1930	Juni 1938	0
Martina N.	1925	1931	6	3	48	ja	1934	März 1938	0
Marianne H.	1931	1937	6	3	13	nein	—	Juni 1938	A
Barbro S.	1927	1933	6	7	25	ja	1933	Febr. 1938	A
Sune M.	1929	1935	6	1	7	ja	1935	Jan. 1938	0
Åke M.	1927	1933	6	2	13	ja	1934	Jan. 1938	0
Vivi B.	1926	1932	6	7	76	ja	1932	Juni 1938	0
Olov G.	1925	1932	7	5	20	ja	1932	Jan. 1937	0
Anna A	1918	1925	7	3	8	ja	1925	April 1936	0
Margareta L.	1921	1928	7	4	16	ja	1928	Jan. 1936	0
John C.	1930	1937	7	4	28	ja	1937	Mai 1938	0
Hubert J.	1922	1929	7	3	16	ja	1930	Juni 1934	0
Greta S.	1926	1933	7	7	28	ja	1933	März 1938	0

Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung? Ursache?	Besserung d. Zustands nach d. Umlegung? In welcher Beziehung?	
begonnen	um- gelegt	abge- schlos- sen			
	ja		nein	ja	Allgemeinzustand erheblich gebessert.
	ja		ja, Angina	ja	Allgemeinzustand jetzt viel besser.
	ja		nein	ja	In jeder Beziehung unerhörter Unterschied; u. a. grössere Widerstandsfähigkeit, bessere Laune.
	ja		nein	ja	Zustand wesentlich gebessert. Mehr Ausdauer, bessere Laune.
	ja		nein	ja	Stimmung besser. Wächst, gedeiht gut.
	ja		nein	ja	Grössere Widerstandsfähigkeit. Vermag jetzt gelegentliche Erkrankungen, welche vorkamen, gut zu überwinden.
	ja		nein	ja	Allgemeinzustand viel besser.
	ja		(ja, Hals- lymphom)	ja	Bessere Entwicklung, freier u. natürlicher. Jetzt keine Insulinbeschwerden.
	ja		nein	ja	Ist jetzt gesund, munter u. stark.
	ja		nein	ja	Besserer Allgemeinzustand, verträgt jetzt alle Speisen.
	ja		(ja, Tonsill- ektomie)	ja	Allgemeinbefinden u. Appetit besser. Ruhiger u. artiger.
	ja		nein	ja	Viel stärker, gedeiht besser.
	ja		nein	ja	Besserer Allgemeinzustand, körperliche Entwicklung besser.
	ja		nein	ja	Stärker.
ja			nein	ja	Kaum wiederzuerkennen. Jetzt munter u. gedeiht wie ein normales Kind.
	ja		nein	ja	Physisch u. psychisch gebessert. Spitzenleistung in der Schule ohne Ermüdung.
nein			nein	nein	Schon vorher munter gewesen.
	ja		nein	ja	Seitdem das Insulin auf die richtige Zeit verlegt wurde niemals Versager.
	ja		nein	ja	In jeder Beziehung besserer Allgemeinzustand.
	ja		nein	ja	Enorme Besserung. Umgebung kann ruhig schlafen. Keine Kosten für Diät.
	ja		nein	ja	Unbeschreiblich. Früher krank, jetzt gesund.
	ja		ja, 1 Woche	ja	Erheblich besserer Allgemeinzustand, Körperkraft gut.
	ja		nein	ja	Viel stärker u. lebhafter.
	ja		nein	ja	Jetzt stark u. munter.
	ja		nein	ja	D. ganze Zeit nach d. Krankenhausaufenthalt gesund gewesen. Wächst besser.



Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung? Ursache?	Besserung d. Zustands nach d. Umlegung? In welcher Beziehung?	
begonnen	um- gelegt	abge- schlos- sen			
	ja		nein	ja	Jetzt gesund u. munter.
	ja		nein	ja	Stärker u. gesünder. Entwickelt sich u. wächst ganz anders.
	ja		nein	ja	Jetzt gesund, gedeiht gut. Dick, rotwan- gig. Ist der Sonnenschein der Familie.
	ja		nein	ja	In jeder Beziehung. Stärker u. »fried- licher«.
	ja		{ ja, 1 Woche, akute Rachen- infektion }	ja	{ Besserer Allgemeinzustand. Keine Insulin- beschwerden. }
	ja		nein	ja	Nicht so nervös u. müde.
	ja		nein	ja	In viel besserer Verfassung.
	ja		ja, Blinddarmop.	ja	Ganzes Befinden besser.
	ja		nein	ja	Stärker, gesünder u. munterer.
	ja		nein	ja	Ruhiger, besserer Gesundheitszustand.
	ja		nein	ja	Kräftiger, keine Insulinbeschwerden.
	ja		nein	ja	Stärker.
	ja		nein	ja	Grössere Abwechslung im Essen u. weni- ger Insulin.
	ja		nein	ja	Viel munterer u. frischer.
	ja		nein	ja	Stärker u. kräftiger.
	ja		nein	ja	{ Nicht so müde. Gesteigerte Arbeitslust u. Energie. }
	ja		nein	ja	{ Allgemeinzustand besser. Jetzt gutgelaunt u. lebhaft. }
	ja		nein	ja	{ In jeder Beziehung! Körperkraft u. All- gemeinbefinden besser. }
	ja		nein	ja	{ Jetzt gesund u. munter wie vor d. Er- krankung. }
	ja		nein	ja	Frischer.
	ja		nein	ja	Erhebliche Besserung in jeder Beziehung.
	ja		ja, Ölit. med.	ja	{ In jeder Weise besser. Gesünder u. mun- terer. }
	ja		nein	ja	Viel besser u. stärker.
	ja		nein	ja	{ Munterer, die Arbeit geht leichter. Frü- her krank, oft Fieber. }
	ja		nein	ja	Stärker und arbeitsfähiger. Leben leichter!

	Ge- burts- jahr	Diabe- tes kon- statiert (Jahr)	Alter beim Aus- bruch d. Krank- heit (Jahre)	Krankenhaus- behandlung wegen Diabetes vor Umlegung d. Behandlung		Insulin- behandlung		Beobach- tungszeit für Umlegung d. Behand- lung	Typus
				Anzahl Male	Gesamt- dauer in Wochen	vor d. Um- legung	seit		
Georg J.	1923	1935	12	8	36	ja	1935	April 1938	0
Birger E.	1918	1930	12	5	20	ja	1930	Jan. 1938	0
Maggie R.	1908	1921	13	9	40	ja	1923	Aug. 1938	0
Valter P.	1922	1936	14	3	36	ja	1936	Febr. 1938	0
Maj L.	1912	1926	14	10	96	ja	1927	April 1937	0
Dick O.	1919	1933	14	6	20	ja	1933	März 1937	0
Katarina N.	1921	1935	14	3	12	ja	1935	Dez. 1937	A
Arline E.	1917	1931	14	8	16	ja	1931	Okt. 1937	A
Elof J.	1919	1933	14	1	8	ja	1935	Juni 1938	0
Ebba J.	1911	1925	14	1	4	ja	1925	(Sept. Okt.) 1938	0
Anna B. S. T.	1925	1933	8	2	7	ja	1937	Aug. 1938	0
Göte K.	1915	1929	14	4	96	ja	1929	Juli 1937	A
Signe W.	1913	1928	15	3	>12	ja	1929	Juni 1937	0
Gunnar N.	1921	1936	15	2	24	ja	1936	Jan. 1937	B
Lars F.	1916	1931	15	4	8	ja	1933	Dez. 1937	0
Brita B.	1921	1936	15	2	20	ja	1937	Febr. 1938	A
Brita S.	1920	1935	15	3	14	ja	1935	März 1938	0
Nils B.	1916	1931	15	2	7	ja	1937	Febr. 1938	B
Bengt Ö.	1915	1931	16	4	11	ja	1931	Nov. 1937	0
Bernhard L.	1921	1937	16	2	14	ja	1937	April 1938	A
Erik F.	1916	1932	16	3	36	ja	1933	Febr. 1937	0
Holger A.	1914	1930	16	6	36	ja	1930	März 1937	0
Tage A.	1920	1936	16	2	6	ja	1937	Jan. 1938	A
Ingvar J.	1916	1932	16	1	7	ja	1932	April 1938	A
Ragnar W.	1915	1931	16	3	24	ja	1931	April 1938	0

Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung?  Ursache?	Besserung d. Zustands nach d. Umlegung?  In welcher Beziehung?	
begon- nen	um- gelegt	abge- schlos- sen			
	ja		nein	ja	Fühle mich jetzt ganz gesund.
	ja		nein	nein	Kein Unterschied.
	ja		nein	ja	{Frischer und stärker. Kein Gedanke da- ran, dass ich krank bin.
	ja		ja, Blinddarmop.	ja	Kräftiger, jetzt munter u. stark.
	ja		nein	ja	{Erheblich stärker, grosse Wendung zum Besseren.
	ja		nein	ja	{Munterer. War nie so wohl wie nach d. Aufenthalt in d. Privatklinik.
	ja		ja, Otit. med.	ja	{Bessere Laune. Schwellung im Gesicht und am Körper verschwunden.
	ja		nein	ja	Viel stärker. Früher stets schwach.
	ja		nein	ja	{Körperkraft hat zugenommen. Durst u. Urinmenge geringer.
	ja		{ja, Beinbruch} {+ Otit. med. }	ja	Munterer, dicker, Herz ruhiger.
	ja		nein	ja	{Bin stärker geworden u. habe zugenom- men.
	ja		nein	ja	{Allgemeinzustand besser. Weniger Insu- lin u. bessere Diät.
	ja		nein	ja	{Grössere Kraft, mehr Gleichgewicht im Gesundheitszustand.
	ja		nein	ja	{Werde nicht müde wie früher. Gesund u. munter.
	ja		nein	ja	{Jetzt gesund u. stark, lebe wie andere Menschen.
	ja		nein	ja	Erheblich stärker.
	ja		nein	ja	Jetzt ganz gesund.
	ja		nein	ja	{Allgemeinzustand im ganzen beträchtlich gebessert.
	ja		nein	ja	{Jetzt gesund u. wie ein normaler Mensch. Guter Einfluss auf d. Stimmung.
	ja		nein	ja	Körperkraft hat zugenommen.
	ja		nein	ja	{Grössere Arbeitsfähigkeit u. gesteigerte Lebenslust.
	ja		nein	ja	Augen besser.
	ja		nein	ja	{Allgemeinzustand besser. Munter u. in Ordnung.
	ja		nein	ja	Stärker.
	ja		nein	ja	Viel stärker.





Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung? Ursache?	Besserung d. Zustands nach d. Umlegung? In welcher Beziehung?	
begon- nen	um- gelegt	abge- schlos- sen			
ja	ja		nein	ja	Kopfschmerzen verschwunden.
	ja		nein	ja	Bessere Laune u. mehr Energie.
	ja		nein	ja	Stärker u. gesünder.
	ja		ja, Pyelitis	nein	Herzbeschwerden bestehen weiter.
	ja		nein	ja	Ganz gesund u. stark. Früher oft müde.
	ja		nein	ja	Allgemeinzustand erheblich besser.
	ja		ja, Lungentbc.	ja	Laune u. Allgemeinbefinden besser.
	ja		nein	ja	Allgemeinbefinden bedeutend gebessert.
			ja, Tonsillenop.	ja	Nicht so müde.
	ja		(ja, 14 T. Bron- chitis)	ja	Erheblich stärker, bessere Laune.
	ja		nein	ja	Körperlich stärker.
	ja		nein	ja	Die schweren Insulinanfälle sind ver- schwunden.
	ja		nein	ja	Viel stärker, arbeitsfähiger.
	ja		nein	ja	Viel fröhlicher u. normaler.
	ja		nein	ja	Jetzt gesund u. stark.
ja	ja		nein	ja	(Gesteigerte Körperkraft. Kopfschmerzen u. Erbrechen völlig verschwunden.
	ja		nein	ja	Jetzt stark u. munter.
			nein	ja	Stärker u. weniger nervös.
	ja		nein	ja	In jeder Beziehung!
	ja		nein	ja	(Nichts so müde wie früher. Zucker viel gleichmässiger.
	ja		nein	ja	(Müdigkeit u. Teilnahmslosigkeit ver- schwunden. Weniger empfänglich für Erkältungen.
	ja		nein	ja	Jetzt gesund u. munter, gute Laune.
	ja		nein	ja	(Kräftiger, Schlaf besser. Fühle mich jetzt wie ein Mensch!
	ja		nein	ja	(Munter und gesund. Körperkraft ist zu- rückgekehrt.
	ja		nein	ja	(Insulinbeschwerden u. alle Gedanken an d. Krankheit verschwunden.

	Ge- burts- jahr	Diabe- tes kon- statiert (Jahr)	Alter beim Aus- bruch d. Krank- heit (Jahre)	Krankenhaus- behandlung wegen Diabetes vor Umlegung d. Behandlung		Insulin- behandlung		Beobach- tungszeit für Umlegung d. Behand- lung	Typus
				Anzahl Male	Gesamt- dauer in Wochen	vor d. Um- legung	seit		
Margareta J.	1911	1934	23	2	10	ja	1934	April 1937	0
Karin M.	1913	1936	23	2	9	ja	1937	April 1938	B
Brita F.	1910	1934	24	2	8	ja	{Jan. 1936}	März 1936	0
Signe S.	1908	1932	24	3	24	ja	1932	Mai 1937	0
Alvar G.	1910	1934	24	2	32	ja	1936	Okt. 1937	A
Elsa N.	1909	1933	24	1	4	ja	1934	Nov. 1935	A
John F.	1876	1901	25	1	4	nein	—	Dez. 1937	B
Hulda F.	1902	1928	26	5	18	ja	1928	Nov. 1937	0
Herbert E.	1905	1931	26	2	12	ja	1931	Okt. 1937	B
Tyra S.	1906	1932	26	1	5	ja	1932	April 1937	0
Sigrid S.	1903	1929	26	3	20	ja	1929	Juli 1937	0
Åke H.	1908	1935	27	2	4	nein	—	Dez. 1937	B
Gudrun M.	1902	1930	28	3	10	ja	1930	Sept. 1937	0
Olov W.	1905	1933	28	3	12	ja	1934	Mai 1937	A
Elsa P.	1908	1937	29	2	12	ja	1937	April 1938	0
Holger N.	1901	1930	29	2	6	ja	1931	Okt. 1936	0
K. (Axel) V.	1902	1931	29	1	5	ja	1931	Jan. 1938	0
K. Erik B.	1895	1924	29	2	8	ja	1928	März 1938	0
Adar W.	1906	1936	30	1	5	nein	—	März 1938	A
Rut S.	1904	1934	30	6	36	ja	1934	Jan. 1938	0
Eva E.	1896	1926	30	3	14	ja	1927	Nov. 1937	0
Folke E.	1906	1936	30	2	5	ja	{Jan. 1937}	Nov. 1937	B
Bengt J.	1898	1928	30	2	11	ja	1928	Febr. 1938	0
Ragnar B.	1892	1923	31	3	18	ja	1936	Dez. 1937	A
Olov N.	1906	1937	31	1	4	nein	—	Jan. 1938	A

Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung?  Ursache?	Besserung d. Zustands nach d. Umlegung?  In welcher Beziehung?	
begon- nen	um- gelegt	abge- schlos- sen			
	ja		nein	ja	{Allgemeinzustand besser. Jetzt ganz ge-
	ja		nein	ja	{sund.
	ja		ja, 1 W.	ja	{Stärker.
	ja		nein	ja	{Physisch u. psychisch. Früher matt und
	ja		nein	ja	{schwach infolge ungenügender Ernährung.
				ja	{Stärker u. munterer. In jeder Beziehung
				ja	{besser.
				ja	{Erheblich stärker.
	ja		ja, Ikterus	ja	{Ruhiger. Vorher ständige Unruhe.
			nein	ja	{In jeder Beziehung. Spannkraft u. Ar-
	ja		nein	ja	{beitsfähigkeit 100%ig wiederehalten.
	ja		nein	ja	{Viel stärker, klarer im Kopf und bei bes-
	ja		nein	ja	{serer Laune.
	ja		nein	ja	{Jetzt gesund u. munter, als ob ich nie
				ja	{Zuckerkrankheit gehabt hätte.
				ja	{Geringere Insulinbeschwerden.
ja	ja		nein	ja	{Psychisch gebessert.
			nein	ja	{Kraft zurückgekehrt. Allgemeinzustand
	ja		nein	ja	{fast ebensogut wie vor der Krankheit.
	ja		nein	ja	{Jetzt gesund mit völlig normaler Körper-
	ja		nein	ja	{kraft.
	ja		nein	ja	{Viel stärker.
				ja	{Munter u. stark, kann jetzt die Haus-
				ja	{haltsarbeit ohne Hilfe verrichten.
	ja		nein	ja	{Mehr Kraft u. Ausdauer bei der Arbeit.
	ja		nein	nein	{Kein Unterschied.
ja	ja		nein	ja	{Allgemeinzustand erheblich besser. Schlaf
			nein	ja	{ruhiger.
	ja		nein	ja	{Bessere Körperkraft u. mehr Ausdauer.
			nein	ja	{Körperlich u. seelisch wie ein ganz an-
				ja	{derer Mensch.
	ja		nein	ja	{Früher nie so gesund gewesen. Nach dem
	ja		(ja, Halsentzündung)	ja	{Aufenthalt in d. Privatklinik nicht
	ja		nein	ja	{einen einzigen Tag krank.
				ja	{Im allgemeinen stärker, kann mich aber
				ja	{zeitweise schwach fühlen.
	ja		nein	ja	{Stärker u. nicht so müde wie vorher.
				ja	{(Geringere Insulinbeschwerden (jetzt sel-
				ja	{ten). Gesteigerte Arbeitsfähigkeit. Grös-
ja			nein	ja	{seres seelisches Gleichgewicht.
			nein	ja	{(Erhöhtes Wohlbefinden, Kraftsteigerung
				ja	{u. Lebensmut.

	Ge- burts- jahr	Diabe- tes kon- statiert (Jahr)	Alter beim Aus- bruch d. Krank- heit (Jahre)	Krankenhaus- behandlung wegen Diabetes vor Umlegung d. Behandlung		Insulin- behandlung		Beobach- tungszeit für Umlegung d. Behand- lung	Typus
				Anzahl Male	Gesamt- dauer in Wochen	vor d. Um- legung	seit		
Oskar L.	1904	1935	31	1	4	nein	—	Jan. 1938	A
Ingrid Å.	1902	1933	31	1	5	ja	1933	Sept. 1937	0
Hanna Ö.	1903	1934	31	2	7	ja	1935	April 1938	A
Ture N.	1902	1933	31	1	4	ja	1933	Okt. 1936	0
Gunnar R.	1902	1933	31	1	4	nein	—	Okt. 1934	A
Edit H.	1900	1931	31	3	9	ja	1931	März 1938	0
Märta S.	1899	1931	32	15	48	ja	1931	Okt. 1938	0
Georg P.	1902	1934	32	1	4	ja	1935	Juli 1937	A
Evert F.	1901	1933	32	1	4	ja	1935	Febr. 1937	0
Ingeborg K.	1899	1931	32	3	16	ja	1931	April 1936	0
Fredrik V.	1896	1929	33	1	6	ja	1930	Juni 1938	0
Paul W.	1901	1934	33	2	4	ja	1934	(Mai 1937) (Mai 1938)	B
Tanno K.	1901	1934	33	2	4	ja	1934	Okt. 1937	B
Birgit E.	1897	1932	35	5	>20	ja	1932	Mai 1935	0
Erland G.	1893	1928	35	2	8	ja	1928	Aug. 1938	0
John A.	1887	1923	36	3	8	ja	1936	Jan. 1938	B
Karl H.	1884	1921	37	2	8	ja	1936	Febr. 1937	B
Anna B.	1894	1931	37	2	7	ja	1932	April 1938	0
Herbert E.	1894	1932	38	4	16	ja	1933	Mai 1938	0
Ebba H.	1892	1931	39	1	4	nein	—	Mai 1937	A
Greta S.	1889	1929	40	6	18	ja	1932	Juni 1938	0
Olga A.	1890	1931	41	5	96	ja	1931	Febr 1935	0

Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung? Ursache?	Besserung d. Zustands nach d. Umlegung?	
begonnen	um- gelegt	abge- schlos- sen		In welcher Beziehung?	
ja			nein	ja	In jeder Weise munterer.
	ja		nein	ja	Sche jetzt aus wie die verkörperte Ge- sundheit.
	ja		nein	ja	Wieder arbeitsfähig. Fühle mich kern- gesund. Meine Bekannten haben mich nie zuvor so gesund gesehen.
	ja		(ja, Magen- krankheit)	ja	In jeder Beziehung besserer Kräftezu- stand.
ja			nein	ja	Allgemeinzustand gebessert.
	ja		nein	ja	Kraft u. Laune besser.
	ja		(ja, 4 T. ) (Influenza)	ja	In jeder Beziehung. Fühle mich wie ein gesunder Mensch.
	ja		nein	ja	Allgemeinzustand enorm gebessert. Nie Müdigkeit; Arbeitsfreude und Lebenslust sind zurückgekehrt.
	ja		nein	ja	Ausserordentlich viel stärker u. in jeder Beziehung besser.
	ja		nein	ja	In jeder Beziehung.
	ja		nein	ja	Erheblich munterer als vorher.
	ja		nein	ja	Gezund u. stärker. Der früher löstige Nackenschmerz verschwunden.
		ja	nein	ja	Allgemeinzustand merkbar besser. Ar- beitsfähigkeit u. Widerstandskraft er- höht.
	ja		ja, Otit. media.	ja	Stärker, gesteigerte Widerstandsfähigkeit gegen Erkältungen.
	ja		nein	ja	Stärker und arbeitsfähiger.
	ja		nein	ja	Laune besser, auch körperliche Besse- rung. Arbeit intensiver ohne Ermü- dung.
	ja		nein	ja	Stärker, ganz gesund.
	ja		ja, Otit. med.	ja	Viel stärker.
	ja		nein	ja	Viel besser. Wie vor der Krankheit. Nerven jetzt vollständig in Ordnung.
			nein	ja	Esse und schlafe wieder wie ein nor- maler Mensch. Zucker und Säure ver- schwunden. 5 kg. zugenommen.
ja			ja, Blinddarmop.	ja	Allgemeinzustand besser.
ja			ja, Beinbruch	ja	In jeder Beziehung.

	Geburts-jahr	Diabetes konstatiert (Jahr)	Alter beim Ausbruch d. Krankheit (Jahre)	Krankenhausbehandlung wegen Diabetes vor Umlegung d. Behandlung		Insulinbehandlung		Beobachtungszeit für Umlegung d. Behandlung	Typus
				Anzahl Male	Gesamtdauer in Wochen	vor d. Umlegung	seit		
Olof K.	1890	1931	41	2	4	ja	1931	Nov. 1935	0
Eskil L.	1883	1925	42	3	6	ja	1936	Juni 1937	A
Sigrid P.	1881	1923	42	1	12	nein	—	Juli 1937	A
Signe A.	1885	1928	43	3	14	ja	1936	Juni 1937	B
John E.	1885	1929	44	3	36	ja	1931	Aug. 1938	0
Anna O.	1879	1923	44	7	42	ja	1935	März 1938	B
Klas O.	1881	1925	44	8	32	ja	1932	Nov. 1935	A
Anna L.	1890	1934	44	4	8	ja	1935	Sept. 1937	B
Edit S.	1888	1933	45	3	17	ja	1934	Juni 1935	A
Herman S.	1884	1929	45	3	> 5	ja	1932	Juni 1938	0
Charles A.	1887	1933	46	2	4	ja	1933	April 1938	0
Hanna J.	1889	1935	46	4	11	ja	(Mai 1936)	Okt. 1936	A
Marta O.	1888	1934	46	6	18	ja	1935	Aug. 1938	0
Konstans L.	1883	1929	46	4	22	ja	1934	Sept. 1937	B
Ester A.	1888	1936	48	4	16	ja	1937	Mai 1938	B
Signe P.	1883	1932	49	1	4	nein	—	März 1938	A
Greta G.	1885	1934	49	2	6	nein	—	Jan. 1936	A
Elsa S.	1879	1928	49	3	12	ja	1930	Juli 1936	A
Simon A.	1882	1931	49	2	18	nein	—	Febr. 1938	A
Lovisa S.	1880	1929	49	2	12	ja	1929	März 1935	A
Hanna N.	1879	1928	49	2	12	ja	1932	Jan. 1937	A
Edvin A.	1885	1935	50	1	4	nein	—	März 1937	B
Maria T.	1880	1930	50	1	7	ja	1935	Jan. 1937	B
Klara A.	1882	1932	50	1	4	nein	—	April 1938	A
Erik J.	1881	1932	51	2	15	nein	—	Jan. 1938	B

Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung? Ursache?	Besserung d. Zustands nach d. Umlegung? In welcher Beziehung?	
begonnen	um- gelegt	abge- schlos- sen			
ja	ja	ja	nein	ja	Bessere Körperkraft.
	ja		nein	ja	Zunehmende Besserung. Im letzten Jahre besonders munter.
			nein	ja	Jetzt munter u. frisch. Mehr Lebens- u. Arbeitslust.
			nein	ja	Fühle mich gesund.
	ja		nein	ja	Bessere Laune u. Arbeitsfähigkeit.
	ja		nein	ja	Stärker u. besser.
	ja		nein	ja	Gesund u. munter. Voll arbeitsfähig.
	ja		nein	ja	Allgemeinzustand u. Arbeitsfähigkeit merkbar gebessert. Langdauernder Ermüdungszustand verschwunden.
	ja		ja, Ikterus	ja	Diese Umstellung von Insulin u. Kost ausgezeichnet für mich.
	ja		nein	ja	Weniger Schweissausbrüche. Arbeitsfähigkeit u. Allgemeinzustand besser.
	ja		ja, Basedowop.	ja	Zunächst besseres Allgemeinbefinden Dann Basedowsymptome (Müdigkeit, Herzbeschwerden u. Nervenschwäche).
	ja		nein	ja	Stärker, habe nicht das quälende Hungergefühl wie vorher.
ja	ja	ja	nein	ja	Gesünder, keine Insulinanfälle.
			nein	ja	Stärker, Allgemeinzustand besser.
			nein	ja	Viel gesünder u. stärker.
			nein	ja	Allgemeinzustand erheblich besser.
			nein	ja	Weniger müde.
	ja		nein	ja	Erhebliche Besserung.
			nein	ja	Im allgemeinen besseres Befinden.
	ja		nein	ja	Allgemeinzustand besser.
ja	ja		nein	ja	Stärker. Grössere Arbeitslust, fröhlicher.
			nein	nein	Herzbeschwerden.
	ja		nein	ja	Gesünder u. stärker in allen Beziehungen.
ja			nein	ja	Stärker.
			nein	ja	Sehr viel besser auf Grund von kräftigerer Ernährung.



	Geburts-jahr	Diabetes konstatiert (Jahr)	Alter beim Ausbruch d. Krankheit (Jahre)	Krankenhaus-behandlung wegen Diabetes vor Umlegung d. Behandlung		Insulin-behandlung		Beobach-tungszeit für Umlegung d. Behand-lung	Typus
				Anzahl Male	Gesamt-dauer in Wochen	vor d. Um-legung	seit		
Gerda N.	1875	1927	52	3	> 8	ja	1928	April 1937	0
B. A.	1883	1935	52	2	8	ja	1937	Juni 1938	B
Olga S.	1877	1930	52	2	5	ja	1933	Jan. 1935	A
August L.	1880	1932	52	6	22	ja	1934	Febr. 1938	0
Emmy D.	1884	1936	52	3	10	ja	1937	März 1938	A
Ida B.	1881	1936	55	2	6	nein	—	Febr. 1938	A
Anna E.	1876	1932	56	3	6	ja	1933	Aug. 1938	A
Carl N. S.	1881	1937	56	2	12	ja	1937	Nov. 1937	A
Erik E.	1879	1936	57	2	14	ja	1936	Okt. 1937	A
Hilma A.	1880	(März 1938)	58	1	6	ja	1938	Aug. 1938	A
Carl O.	1873	1931	58	1	4	nein	—	Okt. 1937	A
Elof L.	1882	1930	58	1	6	nein	—	Mai 1936	A
Anna B.	1864	1925	61	4	28	ja	1928	Aug. 1938	0

Insulinbehandlung			Krankenhaus- aufenthalt nach Umlegung d. Behandlung? Ursache?	Besserung d. Zustands nach d. Umlegung? In welcher Beziehung?	
begon- nen	um- gelegt	abge- schlos- sen			
	ja	ja	nein	ja	{Allgemeines Wohlbefinden erheblich ge- steigert.
			nein	ja	{Keine Insulinbeschwerden.
	ja		nein	ja	{Viel gesünder. Wie ein neuer Mensch.
	ja		nein	ja	{Körperkraft, Arbeitslust und Lebensfreude sind zurückgekehrt.
	ja		nein	ja	{Müdigkeit verschwunden. Verträge alle Speisen besser.
ja		ja	ja, Ikterus 2 W.	ja	{Gesundheitlich besser. Das lästige Jucken hat aufgehört.
			nein	ja	{Grössere Körperkraft.
	ja		ja, Nephrosklerose, Ikterus + Haemorrhagia cerebri	nein	
	ja		nein	ja	{Jetzt gesund und munter, wie bevor ich krank wurde.
	ja		nein	ja	{Fühle mich wohler.
ja		ja	{ja, 2 mal (Gehirnthrombose)	nein	
ja			ja, Parotitis	ja	{Erheblich besser.
	ja		nein	ja	{Stärker.



ANHANG  
ANALYSEMETHODEN



## Analysemethoden.

Bei der physiologisch-chemischen Blut- und Urinuntersuchung ist es sehr wichtig, dass die Analysen möglichst bald nach Entnahme der Probe ausgeführt werden, damit das Fortschreiten von Reaktionsprozessen verhindert wird. Bei grossen Serien ist es jedoch nicht möglich, die Analysen unmittelbar auf die Entnahme folgen zu lassen; man muss in diesem Falle die Proben fixieren. Da mehrere der üblichen Fixiermittel sauer reagieren und infolgedessen empfindliche Substanzen hydrolysieren können, lassen sie sich nicht mit Vorteil verwenden. Der Harnstoff kann z. B. im Diabetikerurin in gewissen Fällen binnen weniger Tage bei Zimmertemperatur quantitativ zu Ammoniak und Kohlensäure hydrolysiert werden. Dabei steigt der pH-Wert des Urins auf 9,2, die pH-Zahl des freien Ammoniaks. Die Anwesenheit von Toluol verhindert die Bakterienwirkung, hemmt aber nicht die Enzymwirkung. Auch die Aufbewahrung der Probe im Kühlschrank verhindert den enzymatischen Zerfall empfindlicherer Substanzen nicht.

Eine brauchbare, einfache Fixiermethode bei physiologisch-chemischen Blut- und Urinuntersuchungen ist die rasche Tiefkühlung der Proben mit Kohlensäureschnee auf  $-80^{\circ}$  und nachherige Aufbewahrung in gefrorenem Zustand in Isoliergefässen mit Kohlensäureschnee, bis man die Analysen ausführen kann.

## Kohlehydratbestimmungsmethoden

### Bestimmung reduzierender Zuckerarten im Blut

Hierbei fand die Akiji-Iwatakesche Modifikation der Blutzuckerbestimmungsmethode nach Hagedorn-Jensen Verwendung (Biochem. Z. 242, 43, 1932). Das Verfahren ist in *Rapports Mikrochemie des Blutes*, Leipzig 1935, ausführlich beschrieben; es wird daher auf die dortige Darstellung verwiesen.

### Spezifische Bestimmung von Hexosen im Blut (Orzinmethode)

Diese Methode gründet sich darauf, dass alle Hexosen oder aus solchen zusammengesetzte Polysaccharide unter dem Einfluss von konzentrierter Schwefelsäure Oxymethylfurfural bilden (vgl. S. 97). Bei Vorhandensein von Orzin kommt dabei eine Rotfärbung zustande, welche kolorimetrisch gemessen werden kann. Im Blute findet sich allerdings auch Glykogen, welches dieselbe Farbe wie Glykose gibt. Um diese Fehlerquelle zu vermeiden wird das Blut wie bei der Bestimmung von reduzierenden Zuckern mit Zinksulfat und Natronlauge versetzt. Das Glykogen, welche im Blut an Eiweiss gebunden ist (*Desmoglykogen*) wird da ausgefällt und stört die Analyse nicht. Das nicht an Eiweiss gebundene dagegen (das *Lyoglykogen*) wird zusammen mit den Hexosen bestimmt; aus diesem Grunde ist der Orzinwert bei Anwesenheit von Lyoglykogen im Blute höher als der entsprechende Reduktionswert (s. Tab. 19 S. 288).

**Ausführung:** Man gibt zu 0,2 ml Blut in einem 1 ml Wasser enthaltenden Zentrifugenglas 2 ml 0,15n NaOH und 2 ml 3,6-%iges  $\text{ZnSO}_4$  und kocht im Wasserbad drei Minuten, worauf das Gemisch scharf zentrifugiert wird. Dann wird die Flüssigkeit in ein graduiertes Messgefäss gegossen. Der Bodensatz wird in 3 ml Wasser gewaschen und von neuem zentrifugiert. Das Waschwasser gibt man zu dem Inhalt des Messgefässes und verdünnt das Ganze auf 8 ml. Von dieser Menge werden Doppelproben von je 3 ml zur Analyse entnommen.

In ein 50 ml fassendes Erlenmeyerkölbchen pipettiert man 3 ml der Probe und setzt 13 ml  $\text{H}_2\text{SO}_4$  (9 ml konz.  $\text{H}_2\text{SO}_4$  + 4 ml  $\text{H}_2\text{O}$ ) sowie 2 ml Orzinlösung (1 g Orzin in 100 ml 10n  $\text{H}_2\text{SO}_4$ ) zu. Gut umrühren! Das Kölbchen wird mit einem Uhrglas od. dgl. bedeckt und in einem Wasserbad mit konstanter Temperatur von 80—81° 20 Minuten lang erwärmt. Der Blindversuch wird mit dem Reagens ohne Hexose angesetzt. Nur reinstes Orzin und Schwefelsäure (*pro analysi*) sind brauchbar. Bei guten Reagenzien wird der Blindwert fast null. Zur Her-

stellung der Eichkurve dient reine Glykose. Ablesung im Stufenphotometer, Schichtdicke 3 cm, Filter S 50 (*Margarete Sörensen*, *Biochem. Zeitschr.* 260, 247, 1933).

### Bestimmung von Glykogen im Blut

Man kocht 0,1 ml Blut zwei Stunden lang mit 1 ml 40%ige KOH in einem Zentrifugenglas. Nach Abkühlung werden 2 ml absoluter Alkohol zugesetzt, dann lässt man die Probe bis zum nächsten Tage stehen, wo sie scharf zentrifugiert wird. Die Flüssigkeit wird vorsichtig dekantiert und weggegossen. Der Bodensatz wird in 1 ml halbgesättigter  $\text{Na}_2\text{SO}_4$ -Lösung aufgelöst und aufs neue mit 2 ml absolutem Alkohol versetzt. Das Gemisch lässt man dann bis zum nächsten Tage stehen, an welchem die Probe wiederum zentrifugiert wird. Das Dekantat wird abgegossen. Der Niederschlag wird in 3 ml  $\text{H}_2\text{O}$  gelöst und in ein 50 ml fassendes Erlenmeyerkölbchen gebracht. Dann verfährt man wie bei der Hexosenbestimmung mit Orzin laut vorstehender Beschreibung. Das Glykogen wird im Laufe der Bestimmung hydrolysiert.

Über Hydrolyse des Glykogens in saurer Lösung vgl. *Sjögren, Nordenskjöld, Holmgren* und *Möllerström*, *Pflügers Arch.* 240, 427, 1938.

### Bestimmung von Glykose im Urin

Der Harn wird mit neutralem Bleiazetat versetzt und filtriert. Das klare Filtrat wird durch Bestimmung der optischen Drehung im Polarimeter auf seinen Gehalt an Glykose hin analysiert. Kontrollen mit Reduktionsbestimmung und mit Hilfe der Oxymethylfurfurolbildung liefern in der Regel übereinstimmende Werte.



## Methoden zur Bestimmung stickstoffhaltiger Substanzen

### Bestimmung des Reststickstoffs im Blut

Man verdünnt 0,1 ml Blut mit 1 ml 10%ige Trichloressigsäure und zentrifugiert scharf. Die überstehende Flüssigkeit wird von dem Niederschlag abgegossen und in ein grösseres Zentrifugenglas gebracht, worauf 1 ml 25%ige  $H_2SO_4$  und 0,5 ml 10%ige Phosphorwolframsäure zugegeben werden. Der neue Niederschlag wird bei 3500 Umdrehungen pro Minute 20 Minuten lang abzentrifugiert. Das klare Dekantat wird dann in ein Verbrennungskölbchen übergegossen. Man fügt 1 ml konzentrierte  $H_2SO_4$  hinzu und erwärmt das Gemisch mit Zusatz einiger Milligramm Kupfersulfat in Substanz. Wenn die Flüssigkeit dunkelbraun oder schwarz ist, werden ein paar Tropfen 30%iges  $H_2O_2$  zugesetzt. Wenn die Lösung bei weiterer Oxydation farblos und klar wird, dann ist sie zur Destillation nach *Parnas* fertig. Die oxydierte Probe wird mit Wasser verdünnt und ein Tropfen Thymolblau zugegeben, worauf sie mit 30%iger NaOH alkalisch gemacht und mit Wasserdampf im Vakuum destilliert wird. Das Ammoniak wird in 1 ml n HCl aufgefangen. Wenn ungefähr 10 ml Wasser in die Vorlage überdestilliert sind, wird die Destillation abgebrochen. Das Destillat wird in einem Messkölbchen auf 15 ml verdünnt und dann mit 1,5 ml Nessler'sches Reagens versetzt. Die Farbe wird in einem Stufenphotometer abgelesen (Schichtdicke 2 cm, Filter S 43). Die Eichkurve wird mit Ammoniumsulfat in bekannter Menge hergestellt. Ist das Reagens nicht von allerbesten Beschaffenheit, so muss man eine Reagenskontrolle ansetzen. Der sich bei dieser ergebende Wert für den Stickstoff im Reagens wird von dem Wert der Probe abgezogen (*Lindberg*, unveröffentlichte Methode).

## Bestimmung des Glutamins im Blut nach Örström

Der Methode liegt der Verlauf der Hydrolysenkurve bei der Freimachung des Ammoniaks aus dem Glutamin bei saurer Hydrolyse zugrunde. Bei der Eiweissfällung müssen Fällungsmittel verwendet werden, welche alle anderen stickstoffhaltigen Substanzen mit Ausnahme des Glutamins mitreissen.

Eine bestimmte Menge Blut (8—10 ml) wird mit 40 ml  $H_2O$  verdünnt. Unter Umrühren werden 6 ml 25%ige Phosphorwolframsäurelösung zugesetzt. Der Niederschlag wird scharf abzentrifugiert. Die klare Flüssigkeit wird in ein 200 ml fassendes Messkölbchen abgegossen. Den Bodensatz wäscht man 2- bis 3mal in 10 ml 2,5%iger Phosphorwolframsäurelösung. Die Waschflüssigkeit wird zu dem ersten Dekantat hinzugefügt, der Niederschlag wird weggeworfen.

Zu der Lösung (Extrakt + Waschflüssigkeit), welche leicht opak sein kann, ohne dass dies die Reaktion stört, gibt man etwa 150 ml Aq. dest. Dann werden unter Umschütteln tropfenweise 9 ml konzentrierte  $H_2SO_4$  zugesetzt.

Die Lösung wird in ein anderes Messkölbchen derselben Grösse filtriert und bis zum Strich mit Wasser verdünnt. Von dieser Lösung misst man Teilmengen von je 20 ml ab, welche 0, 5 und 10 Minuten lang bei  $100^\circ$  im Wasserbade hydrolysiert werden.

Die hydrolysierten Proben werden mit einem Tropfen Phenolphthaleinlösung versetzt und mit 5n KOH bis zu schwach roter Färbung neutralisiert, worauf 5 ml Boratpuffer (pH 9,3) zugegeben werden. Dann wird die Probe im Parnasschen Apparat destilliert und Nesslerisches Reagens zugesetzt. Das durch die Hydrolyse frei gewordene Ammoniak stammt aus dem Glutamin, welches unter den oben angegebenen Bedingungen binnen 5 Minuten quantitativ hydrolysiert wird (Örström, Hoppe-Seylers Zschr. f. phys. Chem. 271, 77, 1941 sowie unveröffentlichte Arbeiten).

## Formoltitrierung von $\text{H}_2\text{N}$

Man versetzt 5 ml Harn mit einigen Kaliumoxalatkristallen und neutralisiert dann mit 0,1n NaOH, mit Phenolphthalein als Indikator. Darauf gibt man 0,5 ml 35%iges Formalin (neutral?) hinzu, wonach die Probe von neuem mit 0,1n NaOH bis zu demselben Umschlag titriert wird. Ausser für Ammoniak gibt die Formoltitrierung auch für Aminostickstoff einen Ausschlag (*Malfatti, Zschr. f. anal. Chem.* 37, 1908).

## Bestimmung des freien Ammoniaks nach Parnas

Zu 1 ml Urin gibt man 10 ml Boratpuffer (pH 9,3) und destilliert mit Wasserdampf im Vakuum. Das überdestillierte

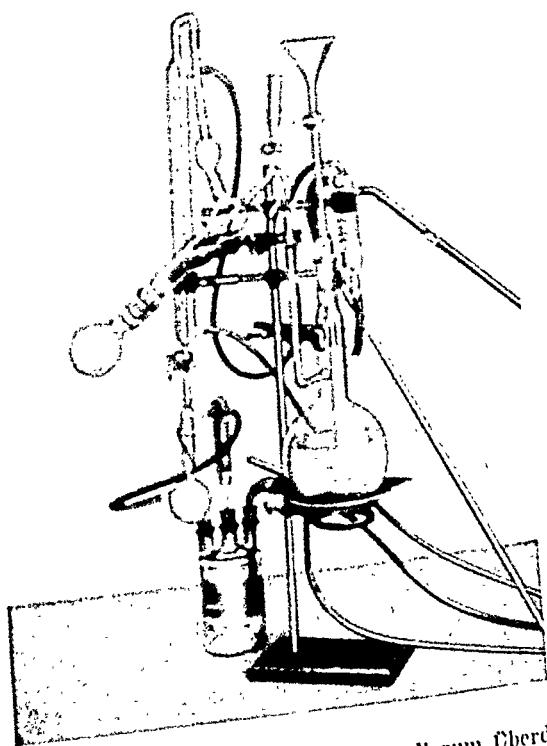


Abb. 107. Destillationsapparat nach Parnas-Teorell zum Überdestillieren von Ammoniak im Vakuum.

Ammoniak wird in 10 ml 0,01n HCl aufgefangen. Die verbrauchte Salzsäure wird mit 0,01n NaOH titriert. Als Indikator wird das *Kjeldahlsche* Reagens verwendet (Lösung a: 0,417 g Methylrot in 835 ml absoluter Alkohol; Lösung b: 0,10 g Methylenblau in 165 ml H<sub>2</sub>O; a und b werden zusammengossen und vor Licht geschützt aufbewahrt).

Boratpuffer: 12,4 g Borsäure + 4,4 g NaOH + 110 ccm H<sub>2</sub>O.

In der Regel ist die Übereinstimmung zwischen dem Ammoniakwert bei Formoltitrierung und dem Ergebnis der Bestimmung nach *Parnas* im Diabetikerharn sehr gut. Manchmal kommen jedoch auf der Ausscheidung von Aminosäuren beruhende Differenzen vor.

### Bestimmung des Glutamins im Urin

10 ml Urin werden mit einem Tropfen Phenolphthaleinlösung versetzt und mit n NaOH bis zur schwachen Rotfärbung neutralisiert, worauf man 10 ml Boratpuffer (pH 9,3) zufügt. Freies Ammoniak, welches die Reaktion stört, wird zuerst im *Parnas*-schen Apparat mit Wasserdampf im Vakuum abdestilliert. Das gesamte freie Ammoniak geht binnen 3 Minuten über.

Der im Destillationskölbchen zurückbleibenden, Glutamin enthaltenden Lösung werden 9 ml konzentrierte H<sub>2</sub>SO<sub>4</sub> zugesetzt, und man bringt das Volumen mit Wasser auf 200 ml. Von dieser Flüssigkeit nimmt man drei Proben von je 20 ml zur Hydrolyse (0, 5 und 10 Minuten) und verfährt wie bei der entsprechenden Blutanalyse.

### Bestimmung von Kreatin und Kreatinin im Urin

Zum Nachweis des Kreatinins im Harn dient die orangefarbene Farbe des Kreatininpikrats.

Das Kreatin wird nach Hydrolyse als Kreatinin bestimmt.

Zur *Kreatinbestimmung* misst man 1 ml filtrierten Urins in einem 100 ml fassenden Messkölbchen ab. Dann werden 20 ml kalt gesättigte Pikrinsäurelösung und 1,5 ml 10%ige NaOH zugesetzt. Das Gemisch wird umgeschüttelt und 20 Minuten

stehengelassen. Danach wird Wasser bis zum Strich aufgefüllt, gemischt und binnen 5 Minuten im Stufenphotometer abgelesen (Schichtdicke 1 cm, Filter S 53). Bei der Bereitung der gesättigten Pikrinsäurelösung muss reinste Pikrinsäure verwendet werden (über die Reinigung vgl. *Rappaport* S. 111). Um eine gesättigte Lösung zu erhalten löst man 1,2 g reine Pikrinsäure in 100 ml kochenden Wassers und kühlt die Lösung ab.

Bei der *Kreatinbestimmung* wird zuerst die Kreatininmenge ermittelt, dann die Summe von Kreatin und Kreatinin nach Hydrolyse, wobei die Differenz aus Kreatin besteht.

10 ml Harn werden mit 0,25 ml konzentrierte HCl drei Stunden im Wasserbade gekocht. Dann wird die Probe in ein 25 ml fassendes Messkölbchen filtriert und bis zum Strich verdünnt. 1 ml dieser Lösung wird zur Kreatininanalyse in der oben angegebenen Weise verarbeitet.

Die Eichkurve wird mit reinem Kreatinin in bekannter Menge hergestellt.

Diese Analysemethode liefert mitunter infolge der Anwesenheit von auf die Farbreaktion störend einwirkenden Substanzen im Harn unbefriedigende Werte. Zur Zeit verfügen wir allerdings über keine bessere, für Kreatin und Kreatinin spezifische Methode.

Enthält der Urin Ketonkörper, so sind diese zuerst durch Destillation mit Wasserdampf, am besten im Parnasschen Apparat, zu entfernen.

### Bestimmung des Cholins im Urin

Das Verfahren gründet sich darauf, dass Cholin mit Jod eine bei neutraler oder schwach saurer Reaktion unlösliche Verbindung eingeht, deren Menge sich jodometrisch bestimmen lässt.

Man versetzt 1 ml der neutralen oder schwach sauren Probe mit 0,3 ml Jodjodkaliumlösung (157,0 g Jod, 200 g Jodkalium, Aq. ad 1000 ml) und zentrifugiert scharf. Die Flüssigkeit wird abgegossen und der Bodensatz rasch und vorsichtig in Eiswasser gewaschen, bis die Jodfärbung verschwunden ist. Dann

gibt man 2 ml Chloroform hinzu und titriert mit 0,01n Natriumthiosulfat, mit Stärke als Indikator. Es ist wichtig, dass man die Probe bei der Titrierung gut umschüttelt, so dass das während der Titration frei werdende Jod aus dem Chloroform in wässrige Lösung übergehen kann (*Roman, Biochem. Zeitschr.* 219, 218, 1930).

## Methoden zur Phosphorbestimmung

### Bestimmung des anorganischen Phosphats im Blut

- Reagenzien:*
- I. 30 ml konzentrierte  $\text{H}_2\text{SO}_4$  werden mit Aq. dest auf 100 ml verdünnt.
  - II. 10 g Ammoniummolybdat, Aq. dest ad 100 ml.
  - III. 1 g Hydrochinon, 20 g Natriumsulfit, Aq. dest ad 100 ml.

*Ausführung:* Man versetzt 0,1 ml Blut mit 1 ml 5%ige Trichloressigsäure und zentrifugiert scharf. Das Dekantat wird in ein kleineres (15 ml fassendes) Messkölbchen pipettiert. Der Bodensatz wird einmal in 1 ml 5%ige Trichloressigsäure gewaschen. Die Waschflüssigkeit wird zum Dekantat hinzugefügt. Etwa 8 ml Aq. dest und danach je 1 ml von Reagens I, II und III werden zugesetzt. Man verdünnt mit Aq. dest bis zum Strich und schüttelt gut um. Nach 30 Minuten Ablesung (blaue Färbung) im Stufenphotometer (Schichtdicke 3 cm, Filter S. 72). Bei Extinktion 1000 in dieser Schichtdicke enthält die Probe  $40,4 \gamma = 0,404 \text{ mg}\%$  P. Der logarithmische Wert wird aus der Eichkurve abgelesen (*Briggs, J. of Biol. Chem.* 53, 13, 1922).

### Bestimmung des Gesamtphosphats im Blut

Man verdünnt 0,1 ml Blut mit 1 ml Aq. dest. und giesst die Lösung quantitativ in ein Reagensglas aus Jenaer Glas oder in ein Kjeldahlkölbchen. Dann setzt man 1,1 ml  $\text{H}_2\text{SO}_4$  (Reagens

I, s. oben) zu und erwärmt das Gemisch. Wenn die Probe schwarz ist, werden einige Tropfen Perhydrol zugegeben und die Oxydation noch etliche Minuten fortgesetzt. Es sollen weisse  $\text{SO}_3$ -Dämpfe abgehen. Ist die Probe klar, so wird sie nach Abkühlung weiterverarbeitet. Ist sie trübe, so setzt man noch einen Tropfen Perhydrol zu, welches abgedampft wird. Es ist wichtig, dass alles Perhydrol beseitigt ist, wenn die Probe zur Analyse verwendet wird.

Der Inhalt des Verbrennungskölbchens wird mit Wasser verdünnt und quantitativ in ein 15 ml fassendes Messkölbchen übergeführt. Danach Zusatz von 1 ml Reagens II und III sowie Auffüllung mit Aq. dest. bis zum Strich, worauf man wie oben verfährt.

Bei der Bestimmung des Phosphors in Blutkörperchen und Plasma wird Heparinblut scharf zentrifugiert und das Plasma von der Blutkörperchenschicht abgehebert. Von dem Plasma und vom Grunde der Blutkörperchenschicht saugt man je 0,1 ml zur weiteren Analyse laut obiger Beschreibung auf.

Organisch gebundenes Phosphat: Differenz zwischen Gesamtphosphat und anorganischem Phosphat.

### **Bestimmung des anorganischen Phosphats im Urin**

Man verdünnt 1 ml Urin mit Aq. dest. auf 10 ml. Von dieser Verdünnung wird 1 ml in ein 15 ml fassendes Messkölbchen pipettiert. Man setzt etwa 10 ml Aq. dest. und dann je 1 ml von Reagens I, II und III zu. Umschütteln und Auffüllen mit Aq. dest. bis zum Strich, darauf Ablesung wie bei der entsprechenden Bestimmung im Blut.

### **Bestimmung des Gesamtphosphats im Urin**

Man verdünnt 1 ml Urin mit Aq. dest. auf 10 ml; von dieser Verdünnung wird 1 ml in ein Reagensglas aus Jenaer Glas oder ein Kjeldahlkölbchen pipettiert. Verbrennung und weitere Untersuchung wie oben für Blut angegeben.

## Methoden zur Chlorbestimmung

### Bestimmung der Chloride im Blut

- Reagenzien:*
- I. Silber-Cer-Reagens. Man füllt 10 ml 0,1n  $\text{AgNO}_3$  in ein 100 ml fassendes Messkölbchen und setzt 20 g Ceriammoniumnitrat, 40 ml konzentrierte  $\text{HNO}_3$  und 15 ml einer in der Kälte gesättigten Lösung von Ferriammoniumsulfat zu. Nach Lösung des Ceriumsalzes wird mit Aq. dest. bis zum Strich aufgefüllt.
  - II. 60%ige Glykoselösung.
  - III. 0,01n Rhodankaliumlösung, aus einer 0,1n KSCN-Stammlösung bereitet.

*Ausführung:* Man bringt mit einer Kapillarpipette vorsichtig 0,1 ml Blut in ein Reagensglas aus Jenaer Glas mit ungefähr 1 ml Aq. dest. ein. Die Pipette wird sorgfältig mit Wasser durchgespült. Dann werden 2 ml  $\text{AgNO}_3$ -Cer-Reagens zugesetzt und das Reagensglas auf 5 bis 10 Minuten in ein kochendes Wasserbad gestellt, bis die Lösung klar gelb wird. Der Überschuss an Ceriumsalz wird durch Zusatz von 0,5 ml Glykoselösung und 2 Minuten langem Kochen im Wasserbad reduziert. Nach Abkühlung titriert man mit KSCN bis zur Orangefärbung. Eine Blindbestimmung wird angesetzt. Die Titer werden gegen gut eingewogene NaCl-Lösung festgestellt (*Rappaport*, Klin. Wschr. 12, 1774, 1933; vgl. *Rappaport*, Mikrochemie des Blutes).

### Bestimmung des Chlors im Urin

- Reagenzien:*
- I. Ferriammoniumsulfatlösung. Zu kalt gesättigter Ferriammoniumsulfatlösung gibt man so viel konzentrierte Salpetersäure, dass die braune Farbe verschwindet.



- II. Kaliumpermanganatlösung. 3,2 g  $\text{KMnO}_4$ , Aq. dest. ad. 100 ml.
- III. Kaliumrhodanidlösung (0,1n KSCN-Lösung). Silbernitratlösung (0,1n  $\text{AgNO}_3$ -Lösung).
- IV. Die Lösungen werden gegen eine gut eingewogene Menge NaCl ausstitriert. Geeignete Konzentration der NaCl-Lösung: 0,5—2 g NaCl auf 100 ml.

*Ausführung:* Man pipettiert 1 ml Harn in ein 25-ml-Messkölbchen. Dann setzt man 1 ml 30%ige  $\text{HNO}_3$  + 4 Tropfen  $\text{KMnO}_4$ -Lösung zur Entfärbung von Urinen mit starker Eigenfarbe zu. Leichte Erwärmung, unter Umständen mit 1 Tropfen Oktylalkohol, um Schaumbildung zu verhindern. Nach der Entfärbung werden 3 ml 0,1n  $\text{AgNO}_3$ -Lösung hinzugefügt. Verdünnung mit Aq. dest. auf 25 ml. Von dem Gemisch werden in ein Erlenmeyerkölbchen 15 ml abgemessen, mit 1 ml Ferriammoniumsulfatlösung als Indikator versetzt und mit 0,1n Kaliumrhodanidlösung auf unverbrauchtes Silbersalz bis zur schwachen Rosafärbung titriert.

## Bestimmung von Kationen

Für die Mikrobestimmung von Kalium, Natrium, Kalzium, Magnesium, Mangan, Eisen und Kupfer hat sich die quantitative Spektralanalyse in der von *Lundegårdh* ausgearbeiteten Form als sehr wertvoll erwiesen. Unter konstanten Versuchsbedingungen wird die sich in wässriger Lösung befindende Probe in einer Zerstäubungskammer mittels eines bei konstantem Luftdruck arbeitenden Injektors fein verteilt und von einem Gasstrom in eine gleichmässig brennende Analysenflamme mitgerissen. Das Licht wird in einem Quarzspektrograph zerlegt und die in Betracht kommenden Spektralbezirke auf einem Film photographiert, der automatisch entwickelt und auch sonst absolut gleichförmig behandelt wird. Der Grad der Schwärzung wird mit einer photoelektrischen Messzelle und einem mit dieser verbundenen Galvanometer

durch die Ausschläge des Lichtzeigers auf einem zweiten Film selbsttätig registriert. Hierdurch werden die Schwärzungsintensitäten der Spektrallinien der Kationen des Blutes in eine Kurve umgeformt, welche mit einer Eichkurve verglichen werden kann. Hinsichtlich Einzelheiten der Methodik wird auf das Spezialschrifttum, in erster Linie auf *Lundegårdhs* Arbeit: Die quantitative Spektralanalyse der Elemente, I u. II, Jena 1929 bzw. 1934 verwiesen, sowie auf eine Veröffentlichung von *Norinder*: Spektralanalytische Untersuchungen über den Kationengehalt des Blutserums unter besonderer Berücksichtigung der Schwangerschaft, Upsala Läkarsällskaps Förhandlingar, Neue Folge 45, 419, 1940.

## Methoden zur Ketonkörperbestimmung

### Bestimmung der Ketonkörper im Blut

Zum Nachweis von Ketonkörpern in 0,1 ml Blut ist eine Methode ausgearbeitet worden, der die Farbreaktion des Azetons mit Salizylaldehyd zugrunde liegt (*Fabinyi*, Chem. Zbl. II, 302, 1900). Diese Methode stimmt in allen wesentlichen Punkten mit der von *Krainick* (Klin. Wschr. 17, 451, 1938) angegebenen überein, welche damals noch nicht bekannt war. Bei Normalwerten im Blut liefern diese Mikroanalysen an kleinen Blutmengen unsichere Resultate, aber beim ketonkörperbildenden Diabetes mit Anhäufung von Ketonkörpern im Blut führt die Methode zu guten Werten. Die Beseitigung von Zucker, Aldehyden und anderen die Reaktion störenden Substanzen findet durch Ausfällung mit Kupfer-Kalk und nachfolgender Destillation statt.

### Bestimmung des Gesamtazetons im Blut

Man pipettiert 0,1 ml Blut in ein Zentrifugenglas, welches 1 ml 10%iges  $\text{CuSO}_4$  enthält. Dem Gemisch setzt man 1 ml 10%ige  $\text{Ca(OH)}_2$ -Suspension zu. Die Probe wird scharf zentrifugiert und von der klaren Flüssigkeit 1 ml zur Analyse ent-

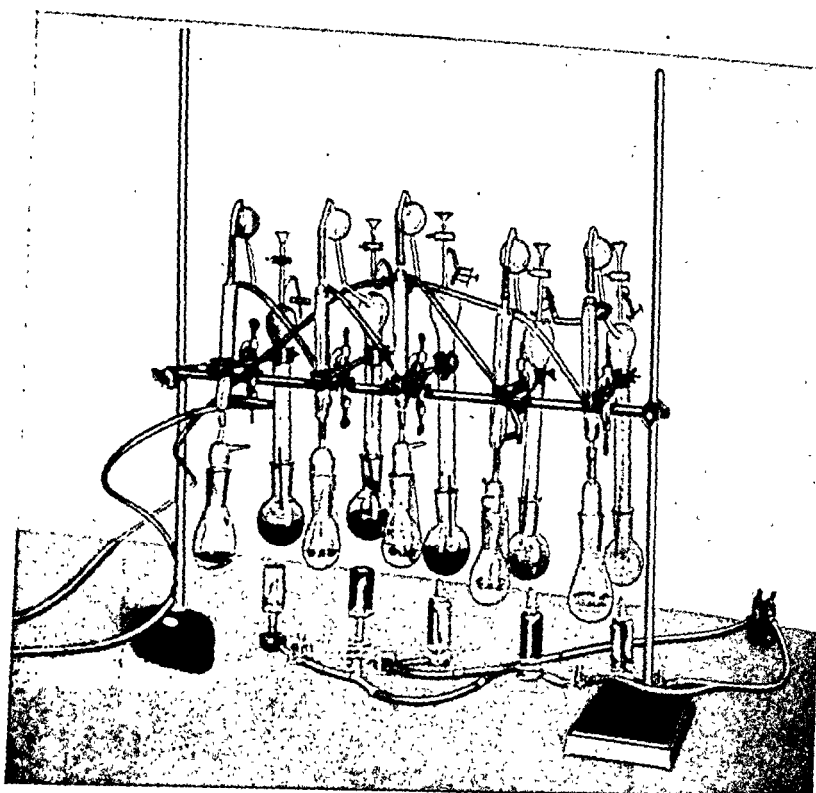


Abb. 108. Apparat nach Lindberg zur Destillation bei Normaldruck oder Vakuum zwecks Mikrobestimmung von Ketonkörpern und Alkohol.

nommen sowie in einen 50 ml fassenden Destillationsapparat mit Trichter und Hahn eingefüllt, letzteres damit man auch während der Arbeit Reagenzien in das Kochkölbchen einbringen kann (NB. Bei Blutanalysen Normalschliff an allen Gefäßen, keine Verbindungsstücke aus Gummi!) In das Destillationskölbchen werden durch den Trichter 5 ml 10%ige Schwefelsäure und 5 ml Wasser gegossen.

Die auf 6 ml graduierte Vorlage ist vorher mit 2 ml 10,5n NaOH und 1 ml 20vol-%igen Salicylaldehyds in 95%igem Alkohol (20 ml Salicylaldehyd, 95%iger Alkohol ad 100 ml) beschickt worden. Die Destillation wird so lange fortgesetzt, bis das Gesamtvolumen des Destillats 6 ml beträgt. Die Vorlage soll eisgekühlt sein. Nach Beendigung der Destillation wird das Destillat im Wasserbad oder Thermostaten bei 50° 70 Minuten lang erwärmt. Die Rotfärbung wird dann im Stufen-

photometer mit dem Reagens als Vergleichslösung abgelesen (Schichtdicke 1 cm, Filter S 50).

Bei dieser Destillation erhält man Azeton und Diazetsäure.

### Bestimmung der $\beta$ -Oxybuttersäure im Blut

Soll auch die  $\beta$ -Oxybuttersäure bestimmt werden, dann wird die Vorlage gegen eine neue, ebenfalls mit 2 ml 10,5 NaOH und 1 ml 20vol.-%iger Salicylaldehyd beschickte ausgewechselt. Durch den Trichter des Destillationsapparats werden jetzt 3 ml Wasser und 10 ml 1%ige Kaliumbichromatlösung zugesetzt. Man destilliert wie zuvor so lange, bis das Gesamtvolumen des Destillats 6 ml beträgt, dann wird die Destillation abgebrochen. Das Destillat wird 70 Minuten lang auf 50° erwärmt und in der oben angegebenen Weise photometrisch untersucht.

Um eine Eichkurve zu erhalten setzt man Proben mit reinen Lösungen von bekannter Konzentration unter gleichartigen Bedingungen an. Die Ausbeute von Azeton aus  $\beta$ -Oxybuttersäure beträgt nur etwas über 50 %, ist aber bei Beachtung der gegebenen Vorschriften konstant (*Lövgren, Lindberg*, nicht veröffentlicht. Vgl. auch *Wretling*, *Acta physiol. Scand.* 3, S. 329, 1941/42).

### Bestimmung der Ketonkörper im Urin

Bei der Bestimmung des Gesamtazetons und der  $\beta$ -Oxybuttersäure im Urin wurde die Lublinsche Mikromethode (*Klin. Wschr.* 1, 894, 1922) angewendet, welche sich auf das Jodbindungsvermögen des Azetons in alkalischer Lösung unter Bildung von Jodoform gründet. Wenn Ketonkörper im Harn auftreten, überwiegen sie mengenmässig so stark, dass andere jodbindende Substanzen die Reaktion nicht in nennenswertem Grade stören, weshalb die Methode für Urin brauchbar ist.

## Bestimmung des Gesamtazetons und der $\beta$ -Oxybuttersäure im Urin

Man füllt 0,5 ml Urin — bei hochgradiger Ketonurie 0,25 ml — in ein 50-ml-Destillationskölbchen und verdünnt mit 25 ml Wasser, worauf 1 ml 10%ige Essigsäure zugesetzt wird. In der Vorlage werden 5 ml 5n NaOH mit 10 ml 0,01n Jodlösung gemischt. Der Destillationsapparat soll so konstruiert sein, dass die kondensierten Dämpfe unter der Oberfläche der alkalischen Jodlösung einfließen (Abb. 109). Nach 10 Minuten langer Destillation wird die Vorlage gegen eine neue ausgewechselt, welche 5 ml 5n NaOH und 15 ml 0,01n Jodlösung enthält. In den mit einem Hahn versehenen Trichter des Destillationskölbchens giesst man 25 ml 0,4n Bichromatschwefelsäure (1050 ml konzentrierte  $\text{H}_2\text{SO}_4$  + 3950 ml  $\text{H}_2\text{O}$  + 102 g  $\text{K}_2\text{Cr}_2\text{O}_7$ ), welche man nach dem Wechseln der Vorlage tropfenweise in das Kölbchen fließen lässt. Dabei wird die  $\beta$ -Oxybuttersäure zu Azeton oxydiert. Die Destillation wird nach weiteren 15 Minuten abgebrochen.

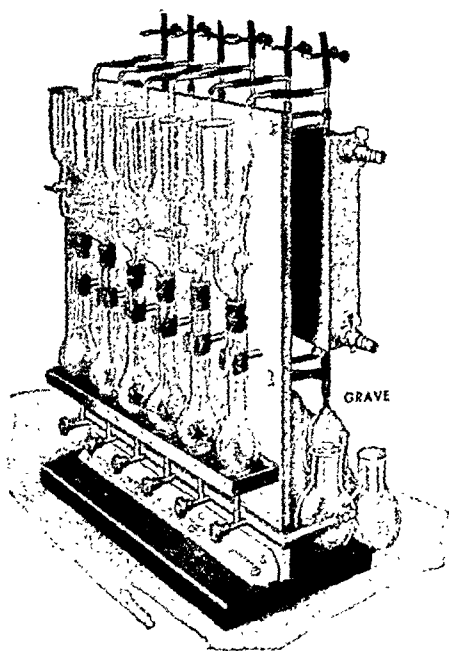


Abb. 109. Destillationsapparat nach Möllerström zur Bestimmung von Ketonkörpern im Urin.

Zu dem Inhalt der Vorlage gibt man 5 ml 5n HCl bis zu saurer Reaktion, wobei Jodoform ausfällt und die Lösung durch den Jodüberschuss braun wird. Dieser Überschuss wird mit 0,01n Natriumthiosulfat bis fast zur Farblosigkeit titriert und dann 1 ml 1%ige Stärkelösung als Indikator zugesetzt. Die Titrierung wird fortgesetzt, bis die blaue Farbe ganz verschwunden ist. Gleichzeitig werden entsprechende Blindbestimmungen ohne Urin vorgenommen. Die verbrauchte Jodmenge wird aus der Differenz zwischen den letzteren und den Proben berechnet.

1 ml verbrauchte 0,01n Jodlösung entspricht 0,0967 mg Azeton und 0,25 mg  $\beta$ -Oxybuttersäure. Von der  $\beta$ -Oxybuttersäure findet man mit dieser Methode etwa 80 % wieder.

## Sonstige Bestimmungsmethoden

### Bestimmung des Alkohols im Blut

Das Wesen der Methode ist die Bindung störender Substanzen an Natriumwolframat und Quecksilbersulfat, unter Umständen bei einer zweiten Destillation an Kalziumhydrat, so dass man den Äthylalkohol allein im Destillat erhält. Dann Oxydation mit alkalischem Permanganat bei 100° und jodometrische Bestimmung des nicht verbrauchten Permanganats (Friedemann-Klaas, Journ. of Biol. Chem. 115, 47, 1936).

- Reagenzien*
- I. Redestilliertes mit Permanganat oxydiertes Wasser.
  - II. 10%  $\text{Na}_2\text{WO}_4$ -Lösung.
  - III. 100 g  $\text{HgSO}_4$  auf 1000 ml 2n  $\text{H}_2\text{SO}_4$ .
  - IV.  $\text{Ca}(\text{OH})_2$ -Suspension (100 g chemisch reines CaO werden mit möglichst wenig Wasser gelöscht, worauf 1000 ml Aq. dest. zugesetzt werden).
  - V. 5n NaOH-Lösung.
  - VI. 0,2n  $\text{KMnO}_4$ -Lösung (man kocht 33 g pro Liter 5–6 Stunden im Wasserbad und fil-

triert mittels Wasserstrahlluftpumpe durch ein Asbestfilter. NB. Die erste Portion wird weggegossen! Von dieser *Stammlösung* werden vor der Verwendung 100 ml auf 1000 ml verdünnt, was einer 0,02n Lösung entspricht.

VII. 10n  $\text{H}_2\text{SO}_4$ .

VIII. Jodkalium pro analysi in Substanz.

IX. Stärkelösung.

X.  $\text{Na}_2\text{S}_2\text{O}_3$  in 0,02n Lösung.

*Ausführung:* Man mischt 0,2 ml Blut, 60 ml Aq. dest., 5 ml 10%ige  $\text{Na}_2\text{WO}_4$ -Lösung, 5 ml  $\text{HgSO}_4$ -Lösung sowie eine kleinere Menge Talk in einem 300 ml fassenden Destillationskölbchen. 30 bis 35 ml werden langsam im Laufe von 15–20 Minuten überdestilliert. Als Vorlage findet am besten ein 100-ml-Kölbchen mit Normalschliff Verwendung (NB. Keine Gummiverbindungsstücke!) Das erste Destillat wird auf ein Gesamtvolumen von 100 ml verdünnt. Hierzu gibt man zur Umdestillation 5 ml  $\text{HgSO}_4$ -Lösung + 5 ml  $\text{Ca}(\text{OH})_2$ -Suspension (bis zu starker Orangefärbung) + eine kleine Menge Talk. 30–35 ml werden nochmals langsam überdestilliert. Das zweite Destillat wird auf 50 ml verdünnt.

Oxydation: In einem 150 ml fassenden Erlenmeyerkölbchen mit einem 100-ml-Becherglas als Haube mischt man:

- 10 ml von dem verdünnten Destillat (aliquoter Teil),
- 15 » Aq. redestillata,
- 10 » 5n NaOH,
- 25 » 0,02n  $\text{KMnO}_4$ -Lösung (unter ständigem Umschütteln zugesetzt).

Sa. 60 ml. NB. Konstantes Gesamtvolumen wichtig!

Die Probe wird im kochenden Wasserbad 20 Minuten erwärmt und dann in fließendem kaltem Wasser gekühlt. Sobald die Probe abgekühlt ist, werden

10 ml 10n  $\text{H}_2\text{SO}_4$  und  
ca. 0,2 g KJ in Substanz  
zugesetzt.

Nach etwa einer Minute weiterer Abkühlung wird das freie Jod mit 0,02n  $\text{Na}_2\text{S}_2\text{O}_3$  austitriert. Wenn die Lösung gelblich ist, gibt man Stärkelösung als Endindikator zu.

Gleichzeitig mit der Probe wird stets eine Blindbestimmung angesetzt.

Berechnung: a ml Thiosulfatlösung bei der Blindbestimmung verbraucht

b	„	„	„	Probe	„
c	„	„	Äquivalent für $\text{KMnO}_4$ .		

Permanganatverbrauch =  $a - b - c$ .

1 ml 0,02n  $\text{KMnO}_4$ -Lösung entspricht 0,0855 mg  $\text{C}_2\text{H}_5\text{OH}$  = 0,00186 Millimol. Bei der Bestimmung reinen Alkohols ist die Ausbeute rund 98,6 %.

### Bestimmung des Alkohols im Urin

Dieselbe Methode kann auch für Urin angewendet werden, wobei 1 bis 2 ml Harn wie vorstehend beschrieben verarbeitet werden. Hier muss indessen nach der ersten Destillation auch eine zweite mit Quecksilberkalk eingeschaltet werden, um die letzten Reste von störenden Substanzen zu beseitigen. Zur Umdestillation nimmt man das erste Destillat und füllt mit Aq. dest. bis zu einem Gesamtvolumen von 100 ml auf. Hierzu setzt man:

5 ml  $\text{HgSO}_4$ -Lösung + 5 ml  $\text{Ca}(\text{OH})_2$ -Suspension (bis zu starker Orangefärbung), ausserdem eine kleinere Menge Talk.

Man lässt 35—40 ml überdestillieren und verdünnt auf 50 ml. Es folgt dann die Oxydation in derselben Weise wie bei der Alkoholbestimmung im Blut.

### Bestimmung des Nikotinsäureamids im Urin

Das Prinzip des Verfahrens ist eine Farbreaktion bei der Sprengung des Pyridinrings mit Bromcyan bei Anwesenheit eines aromatischen Amins (*König*, J. pract. Chem. 69, 105, 1904).



- Reagenzien*
- I. Nikotinsäureamid pro analysi in Substanz zur Bereitung einer Standardlösung (20 mg auf 100 ml H<sub>2</sub>O).
  - II. Phosphatpuffer (850 ml primäres, 150 ml sekundäres Phosphat, pH 6,1).
  - III. Gesättigte Bromzyanlösung. Täglich in für die Analysen des Tages erforderlicher Menge frisch zu bereiten (20 ml gesättigt Br—Aq. dest. + KCN in Substanz bis Entfärbung).
  - IV. Gesättigte Anilinlösung, aus im Vakuum frisch destilliertem Anilin hergestellt (15 ml auf 400 ml Aq. dest.).

*Ausführung:* 25 ml Harn werden durch feinporige Schleichsche Filter in zwei 50-ml-Erlenmeyerkölbchen filtriert. Mit jeder Urinprobe werden zwei Bestimmungen angesetzt, die eine mit Anilin (I), die andere ohne Anilin (II) als Blindversuch.

- I. 10 ml filtrierter Urin  
10 ml Phosphatpuffer  
4 ml Bromzyan  
4 ml Anilinlösung.
- II. 10 ml filtrierter Urin  
10 ml Phosphatpuffer  
4 ml Bromzyan  
4 ml H<sub>2</sub>O.

Probe I und II lässt man nach Zusatz von Anilin bzw. Wasser fünf Minuten lang stehen, worauf die gelbe Farbe im Stufenphotometer abgelesen wird (Schichtdicke 2 cm, Filter S 47).

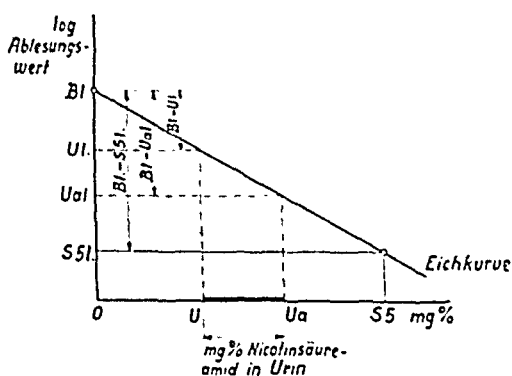
Da sich die Färbung mit der Zeit ändert und die optimale Farbreaktion nach 5 Minuten eintritt, um später abzunehmen, ist es wichtig, dass die Ablesung genau 5 Minuten nach dem Zusatz des Anilins vorgenommen wird. Man darf daher jedesmal nur eine Probe ansetzen und ablesen.

Auch verschiedene Bromzyanlösungen bewirken nicht dieselbe Farbintensität und damit verschiedene Werte für Nikotinsäureamid. Man kann infolgedessen nicht tagaus, tagein dieselbe Eichkurve verwenden, sondern muss eine solche täglich

neukonstruieren. Gleichzeitig mit der Pyridinbestimmung im Urin sind daher täglich noch 3 Proben an einer Lösung mit bekanntem Nikotinsäureamidgehalt (III) sowie eine Nullprobe anzusetzen.

- III. 5 ml Nikotinsäureamidstandardlösung  
 5 ml Wasser  
 10 ml Phosphatpuffer  
 4 ml Bromzyan  
 4 ml Anilinlösung.
- IV. 10 ml Wasser  
 10 ml Phosphatpuffer  
 4 ml Bromzyan  
 4 ml Anilin.

Probe III und IV werden wie die Urinproben nach genau 5 Minuten abgelesen.



*Berechnung nach Thor Lövgren<sup>1</sup>:* Trägt man den logarithmischen Wert für die Nullprobe und die Mittelwerte von 3 Ablesungen für Probe III als Ordinate in einem Koordinatensystem ein, bei dem die Abszisse die Nikotinsäureamidmenge in mg% angibt, so lässt sich beweisen, dass diese Punkte auf einer geraden Linie liegen. Die Kurve der Logarith-

<sup>1</sup> nicht veröffentlicht.

men bildet daher mit der x- und y-Achse ein Dreieck. Jeder Ablesungswert bei sämtlichen mit derselben Bromzyanlösung untersuchten Urinproben muss folglich auf dieser Geraden liegen. Zieht man die Eichkurve täglich durch die sich bei Probe III und IV ergebenden Werte, so erhält man daher mg% Nikotinsäureamid als Unterschied zwischen der Abszisse für die Urinprobe mit Anilin (Ua) und der Abszisse für die Urinprobe ohne Anilin (U) bei der angewandten Bromzyanlösung.

Ua = mg% Nikotinsäureamid im Urin mit Anilin.

U = » » » ohne ».

S5 = » » in 5 ml Standardlösung mit Anilin.

Ual = log Ablesungswert entsprechend Ua.

U1 = » » » U.

S51 = » » » S5.

Bl = » » » Blindbestimmung.

Die Probe enthält  $(Ua - U)$  mg% Nikotinsäureamid.

$$\frac{Ua}{S5} = \frac{Bl - Ual}{Bl - S51}; Ua = S5 \cdot \frac{Bl - Ual}{Bl - S51}.$$

$$\frac{U}{S5} = \frac{Bl - U1}{Bl - S51}; U = S5 \cdot \frac{Bl - U1}{Bl - S51}.$$

$$Ua - U = \frac{S5}{Bl - S51} \cdot (Bl - Ual - Bl + U1) =$$

$$= \frac{S5}{Bl - S51} \cdot (U1 - Ual).$$

$$\text{Nikotinsäureamid im Urin} = \frac{S5}{Bl - S51} \cdot (U1 - Ual) \text{ mg\%}.$$

### Bestimmung der Brenztraubensäure im Blut

Zum Nachweis der Brenztraubensäure diente die Lussche Methode in der Modifikation von Lövgren.

Der Methode liegt die Tatsache zugrunde, dass die Brenztraubensäure mit Dinitrophenylhydrazin ein Hydrazon bildet, welches im Gegensatz zu anderen Hydrazonen in 10%iger  $\text{Na}_2\text{CO}_3$ -Lösung löslich ist; hierdurch lässt sich die Brenztrau-

bensäure leicht von den meisten anderen hydrazonbildenden Substanzen unterscheiden.

*Ausführung:* Man versetzt 0,2 ml Blut in einem Zentrifugenglas mit 1 ml 10%ige Trichloressigsäure. Nach 10 Minuten wird die Probe scharf zentrifugiert. Die klare Lösung wird in ein Reagensglas abgegossen. Der Bodensatz wird mit 1 ml 5%ige Trichloressigsäurelösung aufgeschwemmt, einige Minuten stehengelassen und dann wiederum scharf zentrifugiert. Die überstehende Flüssigkeit wird zu dem ersten Dekantat hinzugefügt. Der Bodensatz wird weggeworfen.

Zu dem gesammelten Dekantat gibt man 1 ml 0,1%iges 2,4-Dinitrophenylhydrazin, in 2n HCl gelöst. Das Gemisch wird mindestens 10 Minuten bei Zimmertemperatur stehengelassen. Dann setzt man 2 ml Äthylazetat zu und schüttelt um, worauf man wartet, bis sich das Gemisch abgesetzt hat. Die obere Äthylazetatschicht enthält Hydrazone und unverändertes Hydrazin. Man saugt sie vorsichtig mit einer Pipette ab (NB. Arbeiten mit Spiegell) und führt sie in ein frisches Reagensglas über. Die zurückbleibende untere Schicht wird mit 1 ml Äthylazetat ausgeschüttelt, dann wartet man wieder, bis sie sich abgesetzt hat und fügt das Äthylazetat zu dem ersten Extrakt hinzu. Die Waschprozedur wird noch ein Mal wiederholt.

Dem gesammelten Äthylazetatextrakt (ca. 4 ml) werden 2 ml 10%ige  $\text{Na}_2\text{CO}_3$ -Lösung zugesetzt, dann mischt man gut durch und lässt das Gemisch wenigstens 3 Minuten stehen. Wenn die Absetzung vollständig ist, führt man die Sodaschicht in ein reines Reagensglas über. Die Extraktion wird zweimal wiederholt, jedes Mal mit 2 ml Sodalösung.

Der gesammelte Sodaextrakt (6 ml) enthält das Dinitrophenylhydrazon der Brenztraubensäure. Die Sodalösung wird beim letzten Mal mit 1 ml Äthylazetat ausgeschüttelt. Der zurückbleibende klare Sodaextrakt, welcher gelbgefärbt sein kann oder nicht, je nach der Menge des verbrauchten Dinitrophenylhydrazins, wird quantitativ in ein neues Reagenzglas übergeführt, welches 4,0 ml n NaOH enthält. Dabei entsteht aus dem Dinitrophenylhydrazon der Brenztraubensäure ein stabiler roter Farbstoff, dessen Konzentration nach 10 Minu-

ten im Stufenphotometer abgelesen wird (Schichtdicke 1 cm, Filter S 53).

Als Vergleichsflüssigkeit wird eine Brenztraubensäurelösung von bekannter Konzentration verwendet. Da sich die Brenztraubensäure in reiner Form leicht verändert, wobei Aldehyde, Ketone und Kondensationsprodukte verschiedener Art entstehen, kann man die gewöhnliche Handelsqualität pro analysi nicht ohne weiteres anwenden, um eine bestimmte Brenztraubensäuremenge zu erhalten. Die Reinigung der Substanz durch Vakuumdestillation ist deshalb nicht angängig, weil sich die Brenztraubensäure dabei in ein dickflüssiges Öl verwandelt.

Lövgren hat für die Darstellung reiner Brenztraubensäure zu Vergleichslösungen folgende Methode ausgearbeitet:

12 ml Brenztraubensäure (Handelsqualität pro analysi) werden mit 5 ml  $H_2O$  versetzt und abgekühlt. Dabei lösen sich Aldehyde und Ketone im Wasser. Das Gemisch wird mit NaCl-Kältemischung auf  $-19^\circ$  tiefgekühlt. Man erhält eine kristallinische, aus Eis und Brenztraubensäure bestehende Masse. Diese wird in einen Büchnerschen Trichter gebracht, mittels einer Wasserstrahlluftpumpe durchgesaugt und rasch in Eiswasser gewaschen. Es bleibt ein Rückstand von feinen, weissen Kristallnadeln übrig, welche ohne Zeitverlust in einem Becherglas gesammelt und mit 50 ml  $H_2O$  versetzt werden. Der Brenztraubensäuregehalt dieser Stammlösung ist nicht bekannt. Man nimmt daher von derselben 5 ml, welche in einem Messkölbchen zur Feststellung des Titors nach Ripper mit Kaliummetabisulfit und Jod auf 100 ml mit Wasser verdünnt werden. Einem mol  $COOH \cdot CO \cdot CH_3$  entspricht 1 mol  $KHSO_3$ .

10 ml 0,4%ige Kaliummetabisulfitlösung werden mit 2 ml verdünnte Stammlösung gemischt und genau 15 Minuten stehen gelassen. Dann wird das unverbrauchte  $KHSO_3$  mit 0,1n Jodlösung ausitiert. Gleichzeitig wird eine Blindbestimmung mit 10 ml Kaliummetabisulfitlösung + 2 ml  $H_2O$  vorgenommen. Aus der Differenz ergibt sich die Brenztraubensäuremenge. Molarer Brenztraubensäuregehalt der Stammlösung

$$= \frac{\text{Differenz} \times \text{Jodtiter}}{4}$$

Mit der Stammlösung wird dann eine Eichkurve zur Berechnung nicht bekannter Brenztraubensäuremengen ermittelt.

### Bestimmung des Brenztraubensäure im Urin

Man versetzt 3 ml Urin in einem Reagensglas (a) mit 2 ml 2,4-Dinitrophenylhydrazin und extrahiert mit 3 ml Äthylazetat. Das Gemisch, welches trübe wird, lässt man 10 Minuten stehen und filtriert dasselbe dann in ein anderes Reagensglas (b). Reagensglas a wird noch zweimal mit Äthylazetat ausgespült, welches durch das Filter geschickt wird. Im Reagensglas b befindet sich einmal eine untere, gelbgefärbte Harnschicht, die Farbstoffe usw. enthält, sodann ca. 9 ml Äthylazetatextrakt mit den gebildeten Dinitrophenylhydrazonen. Der Äthylazetatextrakt wird in ein frisches Reagensglas übergeführt und die zurückbleibende Urinmenge noch einmal mit 3 ml Äthylazetat extrahiert; auch dieser Extrakt kommt zu dem anderen. Nach jeder Extraktion muss die Probe filtriert werden.

Der Äthylazetatextrakt wird dann mit Sodalösung versetzt und sonst wie bei der Brenztraubensäurebestimmung im Blut weiterverarbeitet.

### Bestimmung der Zitronensäure im Urin

Das Prinzip der Methode ist die Bildung von Pentabromazon bei Oxydation der Zitronensäure in saurer Lösung bei Anwesenheit von Brom. Bei Behandlung mit Natriumsulfid in Petrolätherlösung entsteht eine orangefarbene Substanz, welche zur kolorimetrischen Bestimmung (vgl. S. 134) verwendet werden kann (*Pucher, Sherman und Vickery, J. Biol. Chem. 113, 235, 1936*).

*Ausführung:* Man verdünnt 1 ml Urin in einem 150-ml-Becherglas mit 74 ml Wasser und 3 ml 50%ige Schwefelsäure. Das Gemisch lässt man über einem Bunsenbrenner kochen, bis das Volumen etwa 40 ml beträgt. Nach Abkühlung werden 3 ml gesättigte Bromlösung zugesetzt und die Probe 10 Minu-

ten stehengelassen, dann wird sie in einen Scheidetrichter von 100 ml übergeführt. In diesen gibt man 2 ml n KBr-Lösung sowie 10 ml 1,5n  $\text{KMnO}_4$ . Nach 10 Minuten wird das Gemisch mit 3%igen Wasserstoffsuperoxyd entfärbt. Dann wird die Probe mit 10 ml Petroläther extrahiert. *Gut umschütteln!* Man wartet, bis Äther und Wasserschicht deutlich getrennt sind, und hebert die letztere zur weiteren Verarbeitung in ein 100-ml-Kölbchen ab. Der Äther wird noch einmal mit 5 bis 10 ml Wasser durchgeschüttelt, und das Wasser zu der anderen wässrigen Lösung gegeben. Der Äther wird abgelassen. Dann wird die wässrige Lösung in den Scheidetrichter zurückgegossen und in derselben Weise noch zweimal mit 10 ml Petroläther ausgeschüttelt. Der ganze Ätherextrakt wird zusammengegossen und 4 bis 5 Male mit 5—10 ml Wasser durchgeschüttelt. Diese Extraktion ist sehr wichtig und muss sorgfältig ausgeführt werden.

Nun befindet sich die gesamte Zitronensäure in Form von Pentabromazeton im Petroläther.

Der Petroläther wird mit 3 ml frisch bereitete, filtrierte 4%-ige  $\text{Na}_2\text{S}$ -Lösung geschüttelt. Wenn sich das Wasser abgesetzt hat, wird dasselbe in ein 10 ml fassendes Messkölbchen abgelassen, welches mit 3 ml Glyzerin beschickt worden ist. Dasselbe Verfahren wird dann mit 2 ml und schliesslich mit 1 ml  $\text{Na}_2\text{S}$ -Lösung wiederholt. Das Messkölbchen wird nun bis zum Strich mit Glyzerin gefüllt. Innerhalb von 30 Minuten nach Zusatz von  $\text{Na}_2\text{S}$  wird die Färbung im Stufenphotometer abgelesen (Schichtdicke 1 cm, Filter S 47).

Zur Berechnung der Menge wird eine Eichkurve konstruiert, bei der man von bekannten Zitronensäuremengen ausgeht (vgl. *Blomberg und Porjé*, Sv. Läk. tidn. 38, 1002, 1941).

### Bestimmung der $\text{C}_4$ -Säuren des Zitronensäurezyklus im Urin

Bei Störungen in den oxydativen Prozessen beim Diabetes kann das Studium der  $\text{C}_4$ -Säuren des Zitronensäurezyklus aufschlussgebend sein. Die Ausscheidung von Bernsteinsäure, Fumarsäure und  $\alpha$ -Ketoglutar Säure kann man dabei am besten mit der Warburgschen Methodik verfolgen.

## Bestimmung der Bernsteinsäure im Urin

Eine brauchbare Methode gründet sich auf die Sauerstoffaufnahme bei der Oxydation der Bernsteinsäure zu Fumarsäure unter Vermittlung von Sukzinodehydrogenase. Aus der verbrauchten Sauerstoffmenge lässt sich die Menge der Bernsteinsäure berechnen.

**Gewinnung der Sukzinodehydrogenase:** Frisches Rinderherz wird von Fett und Bindegewebe befreit, in Stücke geschnitten und in einer Fleischmühle gemahlen. Die Masse wird in einem grossen Waschbecken mit kaltem Leitungswasser gewaschen, bis letzteres ungefärbt bleibt. Es sind ungefähr fünf Waschungen erforderlich, und das Waschwasser muss jedesmal möglichst restlos entfernt werden. Der ausgewaschene Fleischbrei wird ausgedrückt und mit feinstem Seesand in einem Mörser zu einer Paste zerrieben. Diese wird mittels einer Handpresse ausgepresst. Die Flüssigkeit wird weggeleitet. Zu der Masse gibt man dann etwa 400 ml 5%ige ( $\text{Na}_2\text{HPO}_4 + 12 \text{H}_2\text{O}$ )-Lösung, mischt gut durch, und lässt das Gemisch  $\frac{1}{2}$  Stunden stehen.

Bei der dann folgenden Durchseihung und Pressung der Masse erhält man eine trübe Flüssigkeit mit hohem Sukzinodehydrogenasegehalt. Durch Zusatz von 200–300 ml  $\frac{1}{3}\text{n}$  Azetalpuffer wird der pH-Wert der Flüssigkeit auf 4–6 herabgesetzt. Der Niederschlag wird abzentrifugiert, einmal in Aq. dest. gewaschen, dann mit Kohlensäureschnee gefroren und in diesem Zustand im Vakuum getrocknet. Wenn das gut getrocknete Präparat später im Vakuum aufbewahrt wird, hält es sich ohne nennenswerte Aktivitätseinbusse mehrere Monate. Man hebt es dabei am besten in einem Thunbergschen Röhrchen auf. Vor dem Gebrauch wird das Enzym mit dem zehnfachen Volumen 0,1n Phosphatpuffer (pH 7,4) gemischt. Die homogene Emulsion wird in die Seitenampulle eines Warburgschen Manometers pipettiert, und man lässt sie dann in das Reaktionsgemisch hinabrinnen, wenn sich das Manometer im Wärmegleichgewicht befindet. Die Sauerstoffaufnahme des Enzympräparats ist bedeutungslos (Weil-Malherbe, Bioch. Journ. 31, 301, 1937).



*Extraktion der Bernsteinsäure:* 50 ml Harn werden mit 2n  $\text{H}_2\text{SO}_4$  angesäuert, in einen Kutschner-Streudelschen Extraktor (Abb. 111) gegossen und drei Stunden lang mit frisch destilliertem Äther im Vakuum extrahiert, der vor der Destillation über metallischem Natrium aufbewahrt worden war (NB. Explosionsgefahr mit Peroxyd verunreinigten Äthers bei der Destillation!). Das System wird vor der Extraktion mittels einer Wasserstrahlluftpumpe evakuiert. Nach drei Stunden befindet sich die gesamte Bernsteinsäure in Ätherlösung im Kolben des Extraktors; nun lässt man Luft in den Extraktor einströmen, nimmt den Kolben ab und gibt in diesen 1 ml 0,1n  $\text{Na}_2\text{HPO}_4$ , worauf sämtlicher Äther abdestilliert wird. Die vollständige Entfernung des Äthers ist sehr wichtig, da sonst grosse Fehler entstehen können. Wenn der Äther restlos beseitigt ist, setzt man zu der Lösung 1 Tropfen Phenolrot, worauf die Probe mit n NaOH bis zu einem pH-Wert von rund 7,0 neutralisiert wird. Dann wird die Probe mit Phos-

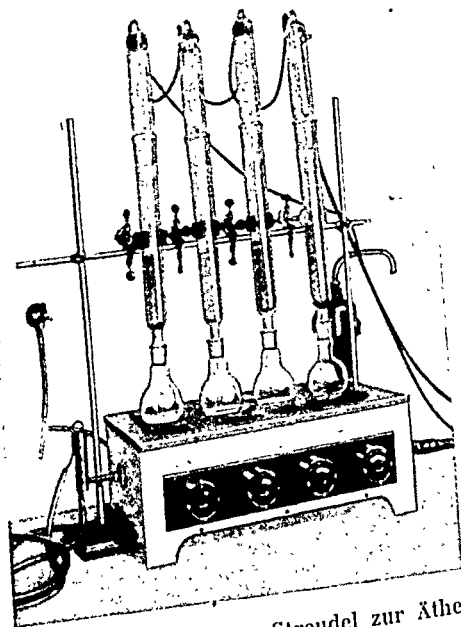


Abb. 111. Extraktor nach Kutschner-Streudel zur Ätherextraktion im Vakuum.

phatpuffer (pH 7,4) verdünnt und ein aliquoter Teil zur Analyse entnommen.

**Manometerbestimmung:** Man verwendet bei dem Warburgschen Manometer konische, mit zwei Seitenampullen und einem Mittelgefäß versehene Becher. Die Seitenampullen sollen je 1—1,5 ml Flüssigkeit fassen.

4 ml des Enzympräparats werden in dem Becher abgemessen. Eine entsprechende Extraktmenge (0,5 — 1,5 ml) kommt in die Seitenampulle, und in das Mittelgefäß gibt man 0,2 ml 2n NaOH. Zu dem Enzym setzt man ausserdem aus der anderen Seitenampulle 0,3 ml Brilliant-Kresylblau als Wasserstoffakzeptor.

Einem mg Bernsteinsäure entspricht ein Sauerstoffverbrauch von 95,1 cmm (*Krebs*, Bioch. Journ. 31, 2097, 1937).

### Bestimmung der Fumarsäure im Urin

Das Prinzip der Methode ist die Reduktion der Fumarsäure zu Bernsteinsäure und Bestimmung der letzteren.

20 ml Harn werden mit 1 g metallisches Zink, 4,6 ml 10n Phosphorsäure und 0,5 ml 20%ige ( $\text{CuSO}_4 + 5 \text{H}_2\text{O}$ )-Lösung versetzt. Nach 60 Minuten ist die Fumarsäure in Bernsteinsäure übergegangen; die letztere wird mit Äther extrahiert und in der oben angegebenen Weise im Warburgschen Manometer bestimmt (*Krebs*, Bioch. Journ. 34, 1041, 1940).

### Bestimmung der $\alpha$ -Ketoglutar Säure im Urin

Dem Verfahren liegt der Umstand zugrunde, dass  $\alpha$ -Ketoglutar Säure bei der Oxydation in Bernsteinsäure umgewandelt wird und sich als diese bestimmen lässt.

Man mischt 10 ml Urin mit 2 ml 1%ige 2,4-Dinitrophenylhydrazinlösung in 10%iger  $\text{H}_2\text{SO}_4$ . Bei starker Azidose wird mehr Phenylhydrazinlösung zugesetzt. Eine Gelbfärbung der Lösung nach der ersten Ausschüttelung ist das Zeichen eines Überschusses an 2,4-Dinitrophenylhydrazin. Vor der Extraktion lässt man das Gemisch 30 Minuten stehen, dann wird es

zweimal mit 5 ml Äther ausgeschüttelt. Der Äther wird abgedunstet, der Bodensatz in 2—5 ml 2n NaOH aufgeschwemmt, in einen etwa 20 ml fassenden Messzylinder gebracht und mit 50%iger  $H_2SO_4$  angesäuert. Dann setzt man 3 ml 2%iges  $KMnO_4$  zu und lässt das Gemisch 15 Minuten lang stehen. Wenn die Farbe verschwindet, fügt man eine kleinere Menge  $KMnO_4$  in Substanz hinzu, so dass die Lösung rot wird. Das Volumen der Lösung wird gemessen und das  $MnO_2$  abfiltriert. Die in Bernsteinsäure umgewandelte  $\alpha$ -Ketoglutaronsäure befindet sich in der Lösung. Sie wird extrahiert und wie oben bestimmt (*Krebs, Bioch. Journ.* 32, 108, 1938).

### Bestimmung der Bernsteinsäure im Blut

Eine empfindlichere und für Blut anwendbare Methode zur Bernsteinsäurebestimmung hat *Sven Forssman* ausgearbeitet (*Acta physiol. scand. Suppl.* V, 1941). Auch bei diesem Verfahren ist das Prinzip die Oxydation der Bernsteinsäure zu Fumarsäure. Statt einer direkten Messung des Sauerstoffverbrauchs wird hier Ferrizyanid als Wasserstoffakzeptor verwendet. Bei der Oxydation entstehen dabei aus jedem Molekül dehydrierte Bernsteinsäure 2 Moleküle Ferrozyanid, welches durch Titrierung mit Ceriumsulfat mit einem geeigneten Indikator gemessen werden kann.

Um die Wasserstoffübertragung von der Bernsteinsäure zu dem Ferrizyanidsystem zu erleichtern, wird ein Zwischenglied mit einem Wasserstoffakzeptor eingeschaltet, dessen Redoxpotential zwischen dem des Ferrizyanidsystems und dem des Bernsteinsäuresystems liegt. Am besten eignet sich hierzu 2, 6-Dichlorophenolindophenol.

### Reagenzien und Lösungen.

**Kaliumferrizyanidlösung:** 0,05molare Stammlösung. 3,292 g Kaliumferrizyanid (Kahlbaum zur Analyse) werden mit Aq. redest. auf 200 ml verdünnt.

**Ferrizyanid-Farbstoff-Puffer-Lösung:** 20 ml Kaliumferrizyanid-Stammlösung + 10 ml 2,6-Dichlorophenolindophenollö-

sung 1:2000 werden mit M/15 Sörensen-Phosphat (pH 7,38) auf 100 ml verdünnt.

*Farblösung:* 0,050 g 2,6-Dichlorophenolindophenol werden in 100 ml Aq. redest. gelöst. Die Lösung muss 24 Stunden bei Zimmertemperatur stehengelassen werden, bevor sie filtriert und verwendet wird. Vor Licht geschützt aufzubewahren!

*M/15 Phosphat (pH 7,38):* 9,501 g  $\text{Na}_2\text{HPO}_4 + 2 \text{H}_2\text{O}$  (Merck, nach Sörensen) und 1,816 g  $\text{KH}_2\text{PO}_4$  (Merck, nach Sörensen), Aq. redest. ad 1 l.

*Mohrsche Lösung:* 0,02molare Stammlösung. 0,7844 Mohrsches Salz (Merck, Ferroammonium sulfuricum pro Analysis + 6  $\text{H}_2\text{O}$ ) + 5 ml 18n Schwefelsäure, mit Aq. redest. auf 100 ml verdünnt. Vor Licht geschützt aufbewahren! 0,001molare Lösung: 5 ml Stammlösung + 5 ml 18n Schwefelsäure werden mit Aq. redest. auf 100 ml verdünnt. Vor Licht zu schützen!

*Indikatorlösung:* 0,10 g Setoglauzin (Setoglauzin salzfrei, I.R. Geigy, S.A., Basel) in 100 ml Aq. dest. gelöst. Vor Filtration und Verwendung 24 Stunden stehenlassen.

*18n Schwefelsäure:* 465 ccm konzentrierte Schwefelsäure (Acidum sulfuricum 1,81 pro analysis Merck) werden vorsichtig mit 535 ml Aq. redest. gemischt.

*Ceriisulfatlösungen:* Etwa 5 g Ceriisulfat (Merck, Cerium sulfuricum oxydatum pro analysis) werden unter Erwärmung in 400 ml 1,8n Schwefelsäure gelöst, dann auf Zimmertemperatur abgekühlt, filtriert und mit Aq. redest. auf 500 ml verdünnt. Der Titer wird mit 0,02molarer Mohrscher Stammlösung festgestellt: 10 ml der letzteren werden in ein Kölbchen pipettiert und mit 3 ml 18n Schwefelsäure, 5 Tropfen Indikator und 20 ml Wasser versetzt. Dann wird mit der Ceriumstammlösung bis zum deutlichen Farbumschlag titriert. Der Titer ist etwa 0,03 molar. 100—200 ml dieser Lösung werden zu ungefähr 0,02molarer Lösung verdünnt. Der Titer wird in derselben Weise wie bei der Stammlösung kontrolliert. 0,001molare Lösung: 5 ml 0,02molare Ceriisulfatlösung + 5 ml 18n Schwefelsäure werden mit Aq. redest. auf 100 ml verdünnt. Diese Lösung muss frisch bereitet sein. Der Titer wird mit 0,001-molarer Mohrscher Lösung bestimmt. Man gibt 2 ccm von

dieser (mit ausgewogener Pipette) in ein Titrierröhrchen und mischt mit 3 ccm 18n Schwefelsäure, 3 ccm Wasser und 3 Tropfen Indikatorlösung. Darauf wird mit 0,001molarer Ceriumlösung aus einer Mikrobürette bis zum deutlichen Umschlagen titriert.

*30%ige Trichloressigsäurelösung:* 30 g Acidum trichloraceticum (Merck), Aq. redest. ad 100 ml.

*0,02molare Natriumsukzinatlösung:* 0,2361 g Acidum succin. pro analysi (Merck) + 40 ml 0,1n NaOH werden mit Aq. redest. auf 100 ml verdünnt. 0,001molare Lösung: 5 ml 0,02 molare Sukzinatlösung, Aq. redest. ad 100 ml. Diese Lösung enthält 118,1  $\gamma$  Bernsteinsäure pro ml. Durch wiederholtes Verdünnen mit gleichen Teilen Wasser erhält man Lösungen mit 59, 29,5, 14,8 und 7,4  $\gamma$  pro ml.

*Aktive Sukzinodehydrogenaselösung:* Wie oben aus Rinderherz oder Pferdefleisch gewonnen.

### A u s f ü h r u n g .

Zu 1 ml der Probe werden in einem Zentrifugenglas 1 ml Ferrizyanid-Farbstoff-Puffer-Lösung und 0,5 ml Ezymlösung gegeben. Die gleichen Mengen werden der Enzym-Wasser-Kontrolle zugesetzt, statt der Probe nimmt man hier jedoch 1 ml Wasser. Es empfiehlt sich, Doppelbestimmungen anzusetzen. Nachdem die Gemische 1 Stunde bei Zimmertemperatur im Dunkeln stehengelassen worden sind, setzt man 0,5 ml 30%ige Trichloressigsäure zu. Nach 10 Minuten wird die Lösung 7 Minuten lang zentrifugiert (3000 Umdrehungen pro Minute). Aus jedem Glase werden (mit ausgewogener Pipette) 2 ml in Titrierröhrchen mit flachem Boden abpipettiert. In jedes Röhrchen kommen 3 ml 18n Schwefelsäure, 3 ml Wasser und 3 Tropfen Indikatorlösung. Dann wird mit etwa 0,001molarer Ceriisulfatlösung aus einer Mikrobürette titriert. Am besten verwendet man hierbei Pressluft zum Durchmischen. Das zu einer Kapillare ausgezogene Ablaufrohr der Mikrobürette muss unter den Flüssigkeitspiegel hinab bis nahe an den Boden neben der Mündung des Pressluftrohrs reichen. Unter gutem Durchmischen lässt man nun die Ceriisulfatlösung langsam in das Titrierröhrchen einfließen, und bei deutlichem Um-

schlagen wird der Hahn der Bürette geschlossen. Im Zweifelsfalle muss man sich davon überzeugen, dass das Umschlagen wirklich erfolgt ist, indem man nach Ablesung der Bürette noch mehr Ceriisulfatlösung zusetzt.

Der Titer der Ceriisulfatlösung wird mit 2 ml 0,001molare Mohrsche Lösung ermittelt, und zwar mit Zusatz von 18n Schwefelsäure, Wasser und Indikator wie oben.

### B e r e c h n u n g.

Der Unterschied zwischen den für die Probe und für die Enzym-Wasser-Kontrolle verbrauchten Mengen Ceriisulfatlösung gibt den Bernsteinsäuregehalt der Probe an. Jedem Molekül dehydrierte Bernsteinsäure entsprechen 2 Moleküle Ferrozyanid und damit 2 Moleküle Ceriisulfat. Einem Verbrauch von 1 ml 0,001molare Ceriisulfatlösung entsprechen also 0,5 ml 0,001molare Sukzinatlösung oder  $\frac{118,1}{2} \gamma$  Bernsteinsäure. Da von 3 ml Reaktionsgemisch zum Titrieren 2 ml abpipettiert werden, muss man die Zahl der Millimeter verbrauchte Ceriisulfatlösung mit  $\frac{2}{3}$  multiplizieren, um die Bernsteinsäuremenge in dem *ganzen* Reaktionsgemisch zu erhalten. Der gefundene Titer der Ceriisulfatlösung ist *etwa* 0,001 molar. Der Ceriisulfatverbrauch muss daher für den entsprechenden Verbrauch einer genau 0,001molaren Lösung umgerechnet werden, was durch Multiplikation mit dem Titerfaktor

$$2$$

bei der Titrierung verbrauchte Ceriisulfatlösung geschieht.

Werden bei der Titerfeststellung a ml Ceriisulfatlösung (zweckmässig Mittelwert von 3—4 Bestimmungen) verbraucht, so ist der schliessliche Faktor also folgender:

$$\frac{3 \cdot 118,1}{2 \cdot 2} \cdot \frac{2}{a} = 88,575 \cdot \frac{2}{a}.$$

Multipliziert man die Anzahl ml Ceriisulfatlösung, die der Sukzinatmenge der Probe entspricht, mit diesem Faktor, so erhält man das Resultat direkt in  $\gamma$  Bernsteinsäure.

Nach Ätherextraktion lässt sich auch die Bernsteinsäure im Harn mit dieser Methode bestimmen.



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# ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM CXLVIII

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## MATHEMATISCHE ERBLICHKEITSANALYSE VON POPULATIONEN

*Von*

GUNNAR DAHLBERG

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MATHEMATISCHE  
ERBLICHKEITSANALYSE  
VON POPULATIONEN



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VON

GUNNAR DAHLBERG

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## Vorwort.

Die menschliche Erbllichkeitsforschung hat vor allem von drei verschiedenen Seiten Impulse empfangen, so dass man zum Teil aus diesem Grunde auf ihrem Gebiete drei verschiedene Forschungsrichtungen unterscheiden kann.

Innerhalb der *Anthropologie und Rassenforschung* ist man allmählich zu der Notwendigkeit gelangt, Vererbung und Milieu von einander abzugrenzen. Nur vererbte Eigenschaften können Rasseigenschaften sein. Für die Rassenforschung hat es sich darum gehandelt, die Ursachen dafür zu ermitteln, warum ein Volk zu Wohlstand und hoher Kultur gelangt und warum ein Volk mitunter verfällt und untergeht. Probleme dieser Art haben in Deutschland zu der Benennung Rassenbiologie geführt, unter welcher jetzt von einigen Seiten nicht nur Untersuchungen über Menschenrassen zusammengefasst werden, sondern auch Untersuchungen, deren Ziel es ist, Populationen unter Berücksichtigung der Vererbung und des Milieus zu analysieren. Hierbei sind Detailuntersuchungen von sehr verschiedenem Charakter notwendig und die Bezeichnung Rassenbiologie hat allmählich die menschliche Erbforschung überhaupt umfasst. Dieser Fachausdruck ist indessen missverständlich. Es handelt sich nicht darum, zu ermitteln, welche Rasse am besten ist. Die Fragestellung ist allgemeiner. Es handelt sich darum, zu erforschen, wie sich die Erbmasse eines Volkes in der einen oder anderen Richtung verändert. Der menschlichen Erbllichkeitsforschung wurde indessen von der Rassenforschung eine soziale Betrachtungsweise und eine grundlegende Fragestellung zugeführt.

Wichtige Impulse wurden auch von der *botanischen und zoologischen Erbllichkeitsforschung* erhalten. Die Lehre von MENDEL wurde für diese Gebiete sichergestellt und weiter ausgebaut und man hat später damit begonnen, diese auch bei Untersuchungen über den Menschen anzuwenden. Anfänglich beachtete man vor allem seltene Eigenschaften. Es ist ja am leichtesten zu zeigen, dass die Gesetze



von MENDEL für derartige Eigenschaften gelten. Allmählich hat sich aber ein überwältigendes Beweismaterial angesammelt und es besteht jetzt kein Anlass, daran zu zweifeln, dass die Gesetze von MENDEL auch für den Menschen Gültigkeit besitzen. Untersuchungen dieser Art sind deswegen jetzt von geringerer Bedeutung. Seltene Eigenschaften haben ja an und für sich mehr Kuriositätswert als Interesse für die Allgemeinheit. Will man weiterkommen, so ist es notwendig, eine Vorstellung über die Rolle zu erhalten, welche die Erblichkeit beim Auftreten gewöhnlicher Eigenschaften spielt.

Die Anwendung statistischer Methoden für die Bearbeitung von Bevölkerungsproblemen hat in einigen Fällen zu Fragen geführt, die Vererbung und Milieu betreffen. Die *biometrische* Schule hat lange versucht, Probleme dieser Art zu lösen, ohne dabei die spezielleren Annahmen zu berücksichtigen, welche der Mendelismus mit sich bringt. Allmählich wurde aber die mendelistische Betrachtungsweise allgemein anerkannt. Von statistischer und biometrischer Seite hat die menschliche Erblichkeitsforschung wichtige Beiträge methodischer Art erhalten und hierdurch auch Mittel, welche Untersuchungen über die Beschaffenheit von Populationen aus Gesichtspunkten der Erblichkeit ermöglichen.

Allmählich ist innerhalb der menschlichen Erblichkeitsforschung eine Richtung entstanden, welche danach strebt, Fragen über die Erbbeschaffenheit von *menschlichen Populationen*, ausgehend vom *Mendelismus* und unter Anwendung von *statistisch-mathematischen Methoden* zu lösen. Die Untersuchungen, die bisher ausgeführt wurden, sind aber verhältnismässig gering an Zahl. Es liegt gegenwärtig keine Arbeit vor, die auf eine zufriedenstellende Weise eine Übersicht über die grundlegenden theoretischen Fragen gibt. Es ist beabsichtigt, in der vorliegenden Arbeit eine solche Übersicht zu geben; in ihr sollen jedoch auch neue Beiträge zur Lösung einiger Probleme veröffentlicht werden. Da es sich um einen ersten Versuch einer synthetischen Darstellung von Erblichkeitsproblemen vom Populationsgesichtspunkt aus handelt, ist es natürlich, dass die Darstellung an den Mängeln leidet, die unausweichlich mit einem solchen Versuch verbunden sind.

Verfasser war durchaus bestrebt, eine Darstellung zu wählen, die auch von Personen ohne mathematische Ausbildung gelesen werden kann. In der Absicht, den Leser allmählich an die Berechnungsmethoden zu gewöhnen, die hier angewendet werden, wurde der

Darstellung besonders im ersten Teil der Arbeit eine sehr elementare Form gegeben, die für mathematisch trainierte Leser störend und langweilig wirken mag. Dies hindert vielleicht nicht, dass andere Leser das Buch möglicherweise schwer lesbar finden. (In diesem Fall kann vielleicht die alte Methode empfohlen werden, zuerst das letzte Kapitel zu lesen, um das Interesse anzuregen.) Es ist auf alle Fälle möglich gewesen, die Darstellung mit Anwendung von elementaren mathematischen Methoden durchzuführen. Die Schwierigkeiten auf diesem Gebiet liegen übrigens nicht so sehr in den mathematischen Berechnungen als in der Begriffsbildung und der Problemstellung.

Die menschliche Erbllichkeitsforschung hat im grossen und ganzen in allzu weitem Ausmass die Methoden und Problemstellungen der experimentellen Erbllichkeitsforschung nachgeahmt. Der Verfasser hofft, dass diese Arbeit zu einer anderen Auffassung und einer auf wesentlichere Fragen gerichteten Umgestaltung der menschlichen Erbllichkeitsforschung bis zu einem gewissen Grad beitragen wird.

Selbstverständlich bestanden besondere Schwierigkeiten, die Literatur, die auf diesem Gebiete vorliegt, zu berücksichtigen, und zwar teils auf Grund der Zeitverhältnisse und teils deswegen, weil Arbeiten über die hier berührten Probleme auf Zeitschriften von sehr verschiedenem Charakter verteilt sind. Die Literaturhinweise, die gegeben werden, sind deshalb sicher unvollständig.

Uppsala april 1943.

*Gunnar Dahlberg.*



## Einleitung.

Die Probleme, die sich vom Gesichtspunkt der Erbllichkeit aus für den Menschen ergeben, können auf zwei verschiedene Fragestellungen zurückgeführt werden:

1. Man kann sich auf den Standpunkt der Bevölkerung stellen und fragen: Was bedeutet eine bestimmte Erbanlage, ein bestimmtes Gen, unter gegebenen Verhältnissen für eine Population und was bedeutet es für die Zukunft? Solche Fragen beziehen sich auf die Frequenz einer Anlage und der zugehörigen Eigenschaften gegenwärtig und in der Zukunft.

2. Man kann sich auf den Standpunkt des einzelnen Individuums stellen und fragen: Wenn eine Person eine bestimmte erbliche Eigenschaft (oder Abstammung) hat, welche erbliche Beschaffenheit besitzen dann ihre Eltern, Kinder oder anderen Verwandten?

Aus beiden Fragestellungen ergeben sich Probleme von grösserer prinzipieller Bedeutung und Fragen, welche sich auf die Methodik der Erbllichkeitsforschung beziehen; es ist selbstverständlich nicht immer möglich eine scharfe Grenze zwischen derartigen Fragen zu ziehen.

Es pflegt hervorgehoben zu werden, dass man bezüglich des Menschen weniger Möglichkeiten besitzt als innerhalb der experimentellen Erbforschung, da man keine Kreuzungen ausführen kann. Dieser Umstand wird bis zu einem gewissen Grade dadurch aufgewogen, dass die Eigenschaften des Menschen gründlicher erforscht sind als die irgend eines anderen Organismus. Ferner wissen wir ziemlich viel über Vorgänge in menschlichen Populationen. Es ist jedenfalls möglich, eine ganze Reihe von Problemen aufzustellen und prinzipiell zu lösen. In vielen Fällen ist es jedoch nicht möglich, eine definitive Antwort zu erhalten. Unsere Kenntnisse über menschliche Populationen sind mangelhaft. Durch die Bearbeitung der Probleme erhalten wir indessen Hinweise, wo wesentlichere Mängel liegen und bekommen dadurch auch Hinweise, die für die weitere wissenschaftliche Arbeit von Bedeutung sind.

In der vorliegenden Arbeit soll nur die erste Fragestellung behandelt werden, die, wie im Vorwort angedeutet, aus der Rassenforschung stammt. Die Aufgabe besteht also darin zu ermitteln, unter welchen Umständen ein Volk im Hinblick auf die erblich bedingten Eigenschaften eine konstante Beschaffenheit besitzt und welche Prozesse Verschiebungen in der Frequenz erblicher Eigenschaften in der einen oder anderen Richtung bedingen können. Bevor wir solche Probleme besprechen, ist es aber notwendig dafür eine Definition zu erhalten, was man darunter versteht, wenn man von erblich- bzw. milieu-bedingten Eigenschaften spricht.

## Einteilung der Eigenschaften vom Gesichtspunkt der Vererbung aus.

Bei lebenden Organismen sind streng genommen alle Eigenschaften durch erbliche Anlagen und gleichzeitig auch durch das Milieu bedingt. Die erblichen Anlagen in einem befruchteten Ei müssen eine bestimmte Beschaffenheit haben, damit das Ei sich während einer längeren oder kürzeren Zeit entwickeln kann. Die Zusammensetzung der Erbmasse kann aber innerhalb gewisser Grenzen variieren. Haben die Anlagen eine vom Durchschnitt der Art zu stark abweichende Zusammensetzung, so kann indessen das befruchtete Ei sich nicht entwickeln oder nur eine kürzere Zeit lang. Das Milieu kann auch nur innerhalb gewisser Grenzen variieren, damit eine Entwicklung stattfinden kann. Weicht das Milieu allzu sehr von dem für die Art normalen ab, so kann keine Entwicklung stattfinden. Anlage und Milieu können also innerhalb der Grenzen variieren, welche der Tod zieht. Die Eigenschaften eines Individuums werden deswegen immer sowohl durch die Anlage als auch durch das Milieu bestimmt. Selbstverständlich ist der Zustand eines Individuums in einem bestimmten gegebenen Augenblick auch vom Alter des Individuums abhängig. Eine bestimmte Eigenschaft ist deswegen streng genommen immer bedingt 1. durch erbliche Faktoren, 2. durch Milieufaktoren und 3. durch einen Altersfaktor.

Bei der Beurteilung der Eigenschaften eines Individuums versucht man oft den Altersfaktor dadurch zu eliminieren, dass man Individuen miteinander vergleicht, die gleich alt sind. Bei einer ganzen Reihe von Fragestellungen vernachlässigt man den Altersfaktor. Besonders wenn es sich um Probleme über Vererbung und Milieu handelt, pflegt man in vielen Fällen stillschweigend den Altersfaktor zu übergehen, ein Vorgehen, das selbstverständlich berechtigt ist, wenn dieser Faktor für die eine oder mehrere spezielle Eigenschaften, um die es sich handelt, ohne Bedeutung ist, oder wenn eine Voraussetzung für die Erwägungen ein Vergleich zwischen Individuen eines

bestimmten Alters ist. Im allgemeinen bespricht man ja die Beschaffenheit von Individuen, die sich in völlig erwachsenem Zustand befinden und bevor Alterserscheinungen sich bei ihnen geltend machen. Es ist nicht exakt zu entscheiden wie lange diese Periode dauert. Man setzt indessen voraus, dass die Grenzen hierbei derart gezogen werden, dass man sich auf der sicheren Seite befindet. Während dieser Periode kann man in den meisten Fällen mit einem gewissen Recht annehmen, dass die Bedeutung des Altersfaktors gering oder überhaupt nicht vorhanden ist. Dies ist jedoch, besonders wenn es sich um Krankheiten handelt, keineswegs immer der Fall. Wenn wir es daher im Folgenden bei den theoretischen Erwägungen im allgemeinen unterlassen, den Altersfaktor zu berücksichtigen, so bedeutet dies eine bewusste Schematisierung. Bei der Lösung eines bestimmten praktischen Problems muss man sich hieran erinnern. Eventuell muss man in einem gegebenen Fall durch eine besondere Untersuchung die Bedeutung der Altersfaktoren festzustellen versuchen.

Wie bereits vorher hervorgehoben, muss man davon ausgehen, dass eine bestimmte Eigenschaft eines Individuums immer sowohl durch Milieu- als auch Erbfaktoren bedingt wird. Welcher Gruppe von Faktoren man die grösste Bedeutung zumisst, hängt von ihrer Frequenz ab. Man nehme an, dass eine Eigenschaft durch einen Erbfaktor und einen Milieufaktor bedingt wird. Ist sowohl der Erbfaktor als auch der Milieufaktor bei allen Individuen vorhanden, so findet sich die Eigenschaft immer. Sie besitzt dann aus Vererbungsgesichtspunkten kein Interesse. Eigenschaften, die bei allen Menschen vorhanden sind, besitzen offensichtlich nur dann Interesse, wenn es sich darum handelt, Unterschiede zwischen Menschen und nahestehenden Tierarten zu besprechen, sind aber selbstverständlich ohne Bedeutung, wenn das Problem Verschiedenheiten zwischen Menschen betrifft. Ist der Milieufaktor immer vorhanden, der Erbfaktor aber nur bei einem Teil der Population, nicht aber bei allen Individuen, so bedeutet dies, dass bei den Individuen, bei denen der Erbfaktor fehlt, ein anderer Erbfaktor vorhanden ist. Die Population zerfällt in zwei Gruppen, zwischen welchen ein erblich bedingter Unterschied vorliegt. Ist hingegen der Erbfaktor bei allen Individuen vorhanden, der Milieufaktor aber nur bei einer Gruppe der Population, so liegt zwischen zwei Gruppen der Bevölkerung ein milieubedingter Unterschied vor. Finden sich in einer Bevölkerung

zwei Erbfaktoren und zwei Milieufaktoren, die verschiedene Verbreitung haben, so muss das Auftreten der Eigenschaft dadurch bedingt werden, dass die Erb- und Milieufaktoren zusammentreffen. Es liegt ein Unterschied zwischen zwei Gruppen vor. Dieser Unterschied wird dadurch bedingt, dass in einer Gruppe ein Erb- und ein Milieufaktor zusammentreffen, während in der anderen Gruppe nur der Erbfaktor oder nur der Milieufaktor vorkommen oder keiner derselben. In diesen letzteren Fällen fehlt die Eigenschaft. (Selbstverständlich ist es auch möglich, dass zwischen diesen letzten drei Gruppen Eigenschaftsunterschiede bestehen.) Von diesen theoretischen Ausgangspunkten aus gelangt man also zu folgender Gruppierung.

*Die erste Gruppe besteht aus erblich bedingten Eigenschaften im eigentlichen Sinne.* Diese Eigenschaften werden durch das Milieu kaum beeinflusst. Für ihre Existenz ist eine bestimmte Kombination von Anlagen erforderlich. Findet sich diese Kombination von Anlagen, so ist die Eigenschaft immer bei den Individuen vorhanden, wie auch das Milieu variiert. Weicht die Beschaffenheit des Milieus von der normalen zu stark ab, so hört sowohl die Existenz der Eigenschaft wie auch des Individuums auf. Die Eigenschaft weist mit anderen Worten keine oder eine geringe durch das Milieu bedingte Variabilität auf, oder auf andere Weise ausgedrückt: die notwendigen Milieufaktoren sind immer vorhanden. Da man nun aber in der Praxis in der Regel niemals weiss, wie sich Individuen mit dieser Kombination von Anlagen unter der Einwirkung aller vorstellbaren Milieufaktoren verhalten, ist es indessen klar, dass eine Behauptung, eine Eigenschaft sei im eigentlichen Sinne erblich bedingt, nur Anspruch auf eine gewisse, begrenzte Sicherheit machen kann. Man kann sich ja vorstellen, dass irgend ein seltener, nicht geprüfter Milieufaktor die Manifestation der Anlagekombination ändern kann. Eine Äusserung darüber, ob eine Eigenschaft im eigentlichen Sinne erblich ist, bezieht sich also nur auf die gewöhnlicheren, für die Art »normalen« Milieufaktoren.

*Die zweite Gruppe besteht aus Eigenschaften, deren Existenz am ehesten dadurch bedingt wird, dass ein oder mehrere nicht allzu gewöhnliche Milieufaktoren vorliegen.* Die Erbanlagen, die vorhanden sein müssen, damit die betreffenden Milieufaktoren das Entstehen der Eigenschaft bedingen, sind bei so gut wie allen Individuen der Art oder Population vorhanden, die man untersucht. Die in



Frage kommenden Milieufaktoren treffen nur einen Teil der Individuen der Population. Unter solchen Verhältnissen sind es die Milieufaktoren, welche die entscheidende Rolle spielen. Ein Beispiel hierfür stellt der Typhus dar. Praktisch genommen haben alle Menschen eine solche Erbbeschaffenheit, dass sie erkranken, wenn sie mit Typhusbazillen angesteckt werden. Wir sagen deswegen, dass die Krankheit durch das Vorkommen des ansteckenden Agens bedingt wird. Streng genommen ist aber auch hier eine bestimmte Erbbeschaffenheit eine notwendige Voraussetzung. Hunde sind z. B. für Typhus unempfindlich. Wird ein Hund mit Typhusbazillen infiziert, so erkrankt er nicht. Er besitzt eine andere Erbbeschaffenheit als der Mensch. Bestimmte Erbanlagen sind also auch für die Existenz dieser milieubedingten Eigenschaften notwendig, da aber die Erbanlagen allgemein vorkommen, spielen sie im Einzelfall nicht die entscheidende Rolle.

Die dritte Gruppe umfasst Eigenschaften, die durch das Zusammenreffen von Milieu- und Erbfaktoren bedingt werden, die keineswegs immer bei den Individuen der Population vorkommen. Die Erbfaktoren verursachen eine bestimmte *Disposition*, eine Neigung gegenüber bestimmten Milieufaktoren auf eine bestimmte Art zu reagieren. Individuen, welchen diese Disposition fehlt, reagieren überhaupt nicht oder kaum, wenn sie der Wirkung der speziellen Milieufaktoren ausgesetzt werden; das Vorhandensein oder Fehlen dieser Milieufaktoren ist für diese Individuen ziemlich gleichgültig. Individuen mit der durch eine spezielle Erbanlage bedingten Disposition reagieren hingegen gegenüber den speziellen Milieufaktoren auf eine abweichende Weise. Wir können solche Eigenschaften als *konstellationell* bedingte ansprechen.

Innerhalb aller Gruppen kann es sich um Eigenschaften handeln, die bei *Gesunden* vorhanden sein können und keine Einschränkung der Lebenstüchtigkeit bedingen, ferner um Eigenschaften, die eine mehr oder minder stark herabgesetzte Anpassungsfähigkeit an die Forderungen, welche das Leben stellt, bewirken. Ist die Herabsetzung der Anpassungsfähigkeit sehr mässig, also so gering, dass das Individuum nur ausnahmsweise durch die Eigenschaft eine Ungelegenheit erfährt, so sprechen wir von *Anomalien*. Ist die Herabsetzung hingegen hochgradiger, so sprechen wir von einer *Krankheit*.

Es ist im übrigen auch klar, dass in allen Gruppen die Eigenschaften entweder akzidentellerer oder permanenterer Natur sein

können. Eine erbliche Eigenschaft ist ja vom Milieu ziemlich unabhängig, ist sie also akzidentellerer Natur, so bedeutet dies nur, dass sie in einem bestimmten Alter eine kürzere Zeit vorhanden ist. Besonders auffällig ist die akzidentelle Natur der erblichen Krankheiten, welche innerhalb einer kurzen Zeit zum Tode führen. Unter milieubedingten Eigenschaften sind ja viele Beispiele für akzidentelle Eigenschaften vorhanden. Hierher kann eine Anzahl ansteckender Krankheiten, wie z. B. Typhus, Masern u. a. gerechnet werden. Zu den permanenteren milieubedingten Eigenschaften können einige Krankheiten, wie z. B. Syphilis, gezählt werden, ferner die Immunität, die im Anschluss an einige akzidentelle, milieubedingte Krankheiten, wie z. B. Masern, auftritt. In der Gruppe konstellationeller Eigenschaften ist der Bedarf nach einer Aufteilung im Hinblick auf die Natur der Eigenschaften, ob sie nämlich akzidentell oder permanent sind, besonders vorhanden. Zu den akzidentellen konstellationellen Eigenschaften gehören eine ganze Reihe infektiöser Krankheiten, für welche die Empfänglichkeit in der menschlichen Population kaum allgemein vorhanden zu sein scheint, z. B. die Encephalitis lethargica und die Poliomyelitis. Wenn man von konstitutionellen Eigenschaften spricht, meint man mitunter rein erbliche, permanente Eigenschaften, das andere Mal konstellationelle, permanente Eigenschaften und manchmal will man mit der Benennung nur ausdrücken, dass man die Eigenschaft als permanent ansieht, ohne eine nähere Aussage zu machen. Der Sprachgebrauch ist mit anderen Worten sehr unbestimmt und wenn möglich sollte man den Terminus vermeiden. Eine konstellationelle Eigenschaft von permanenterer Natur ist z. B. eine derartige Krankheit wie Tuberkulose, die eine bestimmte Disposition, eine bestimmte Erbanlage, sowie ein Milieumoment, nämlich die Tuberkuloseinfektion, voraussetzt und eine Eigenschaft darstellt, die in vielen Fällen eine ziemlich permanente Natur besitzt.

Wie aus dem letzten Beispiel ersichtlich, ist die Grenze zwischen den verschiedenen Gruppen, die wir aufgestellt haben, keineswegs scharf. Ob man eine Eigenschaft als akzidentell oder permanent ansieht, ist ja bis zu einem gewissen Grade eine Geschmackssache. Ferner ist es auch bis zu einem gewissen Grade eine Geschmackssache, ob man der Ansicht sein soll, dass eine bestimmte Kombination von Anlagen so häufig vorkommt, dass ihre Reaktion gegenüber selteneren Milieumomenten Eigenschaften bedingen kann, die

als milieubedingte bezeichnet werden können; es ist auch klar, dass, falls sich ein solcher Anlagetypus gegenüber einem gewöhnlicheren Milieumoment auf eine Weise verwirklicht und gegenüber einem selteneren Milieumoment auf eine andere Weise, so ist es auch bis zu einem gewissen Grade eine Geschmackssache, ob die eine oder die andere Eigenschaft als abnorm bzw. normal angesehen werden soll.

Handelt es sich um Milieumomente, so kann man selbstverständlich auch diskutieren, ob das Milieumoment ausreichend häufig vorkommt, damit das Hauptgewicht auf das erbliche Moment gelegt werden kann. Nimmt man an, dass die progressive Paralyse ihre Grundlage in einer erblich bedingten, speziellen Reaktionsweise hat, so kann man doch sagen, dass die Krankheit insoweit milieubedingt ist, dass nur die, welche mit Syphilis infiziert werden, eine Paralyse bekommen. In einer Bevölkerung, in welcher die Syphilis sehr verbreitet ist, so dass fast jeder Mensch angesteckt wird, würde man indessen Anlass haben, die Krankheit als erblich bedingt anzusehen.

Zusammenfassend soll hervorgehoben werden, dass also bei der Klassifizierung von Eigenschaften die Frequenz der Erb- und Milieufaktoren, die eine Rolle spielen, eine wesentliche Bedeutung dafür besitzt, wie die Eigenschaft rubriziert wird. Haben sowohl Erb- als auch Milieufaktoren für die Entstehung einer Eigenschaft Bedeutung und kommen beide Arten von Faktoren in mässiger Frequenz vor, so bezeichnet man die Eigenschaft als konstellationell. Kommt das Milieumoment sehr häufig vor und ist es bei fast der gesamten Bevölkerung vorhanden, so bezeichnet man die Eigenschaft als erblich im eigentlichen Sinne. Besitzt hingegen die Erbanlage eine sehr hohe Frequenz und die Milieufaktoren weisen eine niedrige Frequenz auf, so ist man der Ansicht, dass die Eigenschaft milieubedingt ist. Kommen sowohl die Erb- als auch die Milieufaktoren in der ganzen Population vor, so haben ja alle Individuen die Eigenschaft und es sind dann keine erblichen Unterschiede innerhalb der Bevölkerung vorhanden; verschwinden die Erb- und Milieufaktoren, so verschwindet ja auch die Eigenschaft aus der Population.

Das System, das wir aufgestellt haben und die Grenzen, die wir gezogen haben, sind offensichtlich bis zu einem gewissen Grade willkürlich. (Die hier angewandte Einteilung der Eigenschaften von dem Gesichtspunkt der Erbllichkeit und des Milieus aus wurde vom Verf. vor kurzem angegeben (DAHLBERG 1939 a) und ist also nicht allgemein anerkannt.) Ob das System und die Terminologie, auf die wir dieses

System aufgebaut haben, in der Praxis anwendbar sind, ist eine Angelegenheit, auf welche die Zukunft Antwort geben wird. Um die Rolle der Erblichkeit sowie die Bedeutung des Milieus klarzulegen, ferner um einen differenzierteren Griff um die Problemstellung zu erhalten, dürfte das System aber gegenwärtig seinen Platz behaupten.

## Der Rassenbegriff und die Mendelschen Erblchkeitsgesetze.

Wie im Vorwort hervorgehoben, hat die medizinische Erblchkeitsforschung von der Rassenforschung Impulse erhalten und von dieser Richtung eine grundlegende Fragestellung bekommen. Es ist deswegen motiviert zu versuchen, den Rassenbegriff vom mendelistischen Gesichtspunkt aus näher zu analysieren.

Man wendet die Bezeichnung Rasse auf eine unklare Weise an und bespricht Rassenfragen auf dieselbe Weise. Definiert man den Begriff Rasse, so pflegt man sich damit zu begnügen hervorzuheben, dass eine Rasse eine Gruppe von Individuen ist, die sich im Hinblick auf die erblich bedingten Eigenschaften von anderen Gruppen unterscheidet. Diese Definition erscheint vielleicht auf den ersten Blick als klar und eindeutig, sie ist aber in der Wirklichkeit in vieler Hinsicht äusserst unbestimmt.

Der Rassenbegriff ist älter als der Mendelismus und das hat dazu geführt, dass bei der Ausbildung des Rassenbegriffes die Anschauungen über Erblchkeit verwendet wurden, die vor MENDEL vorhanden waren. Diese älteren Anschauungen waren niemals klar definiert. Man setzte aber damit fort, diese kaum durchdachten Anschauungen lange nachdem anzuwenden, als der Mendelismus anerkannt wurde, aber in gewissem Ausmasse hat man sich gleichzeitig auch mendelistischer Vorstellungen bedient. Dies hat es mit sich gebracht, dass das Wort Rasse dazu angewandt wurde, sehr unklare und dehnbare Begriffe zu umschliessen.

Die oben angeführte Definition muss ihrerseits in drei Hinsichten näher definiert werden.

Vor allem muss man Klarheit darüber erhalten, was man unter einer *erblich bedingten Eigenschaft* versteht. Im Vorhergehenden haben wir diese Frage besprochen und aus der begriffsmässigen Analyse, die ausgeführt wurde, ergibt sich, dass man nicht nur zwischen erblich bedingten und milieubedingten Eigenschaften unterscheiden

kann. Offensichtlich können konstellationelle Eigenschaften in der Masse, in welchem Erbfaktoren eine Rolle für solche Eigenschaften spielen, Rasseneigenschaften sein.

Diese Seite des Problems besitzt hingegen eine geringere Bedeutung. Wichtiger ist, dass man innerhalb der Rassenforschung, wie bereits vorher hervorgehoben, von vormendelschen Anschauungen über die Natur der Vererbung ausgeht. Handelt es sich darum, die Natur der Materie zu analysieren, so kann man zwischen zwei Annahmen wählen. Man kann annehmen, dass die Materie aus homogenen Substanzen aufgebaut ist, die verschiedene Natur haben können und sich auf verschiedene Weise miteinander vermischen können. Innerhalb der Naturwissenschaft hat man lange mit einem solchen Begriff der Materie gearbeitet. Erst als man annahm, dass die Materie aus kleinen Teilen, Molekülen, Atomen u. s. w. aufgebaut ist, die sich nicht teilen können, ohne zerstört zu werden, konnte man zu einer Übereinstimmung zwischen den experimentellen Ergebnissen und den theoretischen Anschauungen gelangen. Handelt es sich um die Natur der Erbmasse, so hat man zwischen denselben Alternativen zu wählen gehabt. Vor MENDEL nahm man an, dass die Erbmasse eine Substanz ist. Bei den reinen Rassen besass diese Erbsubstanz eine bestimmte Beschaffenheit und bei Kreuzungen zwischen verschiedenen Rassen entstanden Mischrassen auf dieselbe Weise wie man durch Vermischung verschiedener Metalle Legierungen von verschiedenem Charakter erhielt. Kreuzt man einen Neger mit einem Weissen, dürfte das Ergebnis eine Mischrasse, Mulatten, sein. Kreuzt man Mulatten, so müssten die Nachkommen aus demselben Anlass Mulatten sein, wie man bei der Vermischung von zwei Portionen einer Legierung dieselbe Legierung wie vorher erhält. Dass die Nachkommen der Mulatten variieren, stimmt nicht mit dem überein, was man theoretisch zu erwarten hatte.

Der Mendelismus stellt deswegen einen bedeutenden Fortschritt dar, da man jetzt eine Übereinstimmung zwischen Theorie und Empirie erhielt. MENDEL nahm, wie bekannt, an, dass die Erbmasse aus kleinen Teilen, Genen, besteht, die sich auf verschiedene Weise kombinieren können.

Auf dem Gebiete der Rassenforschung wendet man indessen immer noch in einem gewissen Ausmasse die vormendelsche Betrachtungsweise an. Man setzt voraus, dass es reine Rassen gegeben hat. Man ist der Ansicht, dass die Rassenbeschaffenheit bei der Beurteilung

des einzelnen Individuums von sehr grosser Bedeutung ist. Die Individuen können selbstverständlich Verschiedenheiten aufweisen, ihrem innersten Wesen nach sind aber Individuen derselben Rasse doch gleich. Man erörtert diese Frage, als ob es sich um Gegenstände aus bestimmten Metallen handeln würde, die auch eine verschiedene Beschaffenheit haben können und als Küchen- oder Ziergegenstände angewandt werden, ihrem innersten Wesen nach aber doch als gleich angesehen werden müssen. Sowohl die Annahme ursprünglich reiner Rassen, wie die Annahme, dass die Rassenbeschaffenheit immer von fundamentaler Bedeutung ist, sind unrichtig.

Die Individuen innerhalb einer Rasse variieren in hohem Grade. Zwei Menschen weisen ja niemals völlig denselben Genotypus auf. Individuen, die im Hinblick auf ihre Abstammung verschiedenen Rassen angehören, können im Hinblick auf die Beschaffenheit ihrer Anlagen einander ähnlicher sein als Individuen, die derselben Rasse angehören. Zwei normal begabte Individuen, die verschiedenen Menschenrassen angehören, sind in entscheidenden Punkten einander ähnlicher als eine normal begabte Person und ein erblich bedingter Idiot, die derselben Rasse angehören. Zwei Rassen können erblich bedingte Unterschiede im Hinblick auf bestimmte Gene aufweisen, so dass die Gene bei der einen Rasse fehlen und bei der anderen vorhanden sind. Die Unterschiede können ferner einen relativen Charakter haben und dies bedeutet, dass bestimmte Gene eine verschiedene Frequenz innerhalb beider Rassen aufweisen.

Um Rassenunterschiede erschöpfend feststellen zu können, müssten wir bei jedem Individuum innerhalb beider Gruppen, die wir miteinander vergleichen, die Beschaffenheit der Gene kennen. Wir besitzen nur äusserst mangelhafte Kenntnisse und aus diesem Grunde müssen die Aussagen, die über die Rassenbeschaffenheit gemacht werden können, eine sehr begrenzte Reichweite haben. Es sind indessen ohne Zweifel zwischen den verschiedenen Gruppen von Individuen deutliche Unterschiede vorhanden. Wir müssen für diese Unterschiede eine Bezeichnung haben. Es ist deswegen berechtigt von Rassenunterschieden zu sprechen. Macht man aber Aussagen, so muss man sich ständig daran erinnern, dass man nur etwas über bestimmte untersuchte Eigenschaften behaupten kann und dass man auf Grund dessen, was man weiss, keine weiteren Unterschiede konstruieren darf, von denen man nichts weiss. Es kann unnötig erscheinen, dies hervorzuheben. Gerade deswegen, weil man die vor-

mendelsche Betrachtungsweise anwendet, ist indessen eine starke Versuchung vorhanden, über das hinauszugehen, was man wirklich weiss. Stellt man zwischen zwei chemischen Substanzen einen Unterschied fest, so hat man Anlass zu vermuten, dass mehrere Unterschiede vorliegen werden. Aus diesem Grunde ist man vom vormendelschen Ausgangspunkt aus in Versuchung, falls man zwischen zwei Gruppen einen Unterschied festgestellt hat, auch anzunehmen, dass weitere Unterschiede vorliegen; dies stimuliert zu Spekulationen, die zu einer Überwertung der Bedeutung von Rassenunterschieden führen.

Es ist ferner notwendig zu untersuchen, was man unter einer *Gruppe von Individuen* versteht, wenn man den Rassenbegriff definiert. Man ist nicht ohne weiteres der Ansicht, dass die langen Personen eines Volkes einer Rasse angehören und die kleinen einer anderen, trotzdem man sagen kann, dass beide Arten Gruppen darstellen und beide erblich bedingte Unterschiede aufweisen. Dies beruht darauf, dass Individuen von verschiedener Körperlänge sich immer mit einander vermischt haben. Unter einer Gruppe versteht man in Wirklichkeit, wenn man von Rassen spricht, eine Gruppe von Personen, die sich seit langem hauptsächlich miteinander, aber nicht oder weniger häufig mit Personen der anderen Gruppe, mit der man vergleicht, vermischt haben. Die Gruppe soll in Wirklichkeit ein Isolat oder eine Anzahl von Isolaten darstellen, die entweder geographisch oder sozial abgegrenzt sind.

Schliesslich wird in der Definition angegeben, dass die beiden Gruppen verschiedene Eigenschaften aufweisen können. Man fragt sich nun, wie *viele* Eigenschaften erforderlich sind, um von einer besonderen Rasse sprechen zu können. Der kleinste Unterschied, der vorliegen kann, ist auf eine einzige Eigenschaft zurückzuführen; theoretisch gesehen ist es also ausreichend, wenn man im Hinblick auf eine Eigenschaft einen Unterschied feststellen kann, um berechtigt zu sein von einer bestimmten Rasse zu sprechen. Unter solchen Umständen gelangt man also zu folgender Definition (vgl. DAHLBERG 1941):

*Eine Rasse stellt ein Isolat oder eine Gruppe von Isolaten von Individuen dar, die wenigstens einen erblich bedingten absoluten oder relativen Unterschied gegenüber anderen Isolaten aufweisen.*

Eine Eigenschaft ist mit anderen Worten eine Rasseneigenschaft, wenn sie bei einem Isolat oder einer Gruppe von Isolaten vorkommt,



aber in irgend einem anderen Isolat fehlt oder in einer anderen Frequenz vorhanden ist. Streng genommen soll man nicht über Rasseneigenschaften sondern Rassenunterschiede sprechen. Es ist ferner klar, dass die Rassenunterschiede eine sehr verschiedene Größenordnung haben können, weswegen es erwünscht ist, einen Masstab für die Rassenunterschiede zu erhalten. Diese Frage wird später in dieser Arbeit behandelt werden. Es ist nämlich notwendig, ehe dieses Problem behandelt wird, die Mechanismen näher zu besprechen, die Verschiebungen in der Erbmasse bei einem Volk bedingen können.

Im Folgenden wird eine gewisse Kenntnis der Grundzüge der allgemeinen Erblchkeitslehre vorausgesetzt. Die Formeln, die im folgenden aufgestellt werden, bauen sich also auf mendelistische Ausgangspunkte auf.

## Verschiedene Formen von Vererbung.

### Monohybride Diallelie.

Haben wir nur eine Art von Genen, die in einem Anlagepaar vorhanden sind, so werden alle Individuen einer Bevölkerung Homozygoten und einander im Hinblick auf die Vererbung gleich sein. In einer solchen Bevölkerung werden die Unterschiede zwischen den Individuen nur durch eine Variabilität bedingt, welche das Milieu verursacht. Bei monohybrider Diallelie werden hingegen die betreffenden Eigenschaften durch zwei verschiedene Gene bedingt (Diallelie), die einem allelen Paar angehören (Monohybridität). Wir bezeichnen diese Gene mit *R* und *D*. Die Gameten, die gebildet werden, können von zweierlei Art sein, und zwar teils Gameten, die *R*-Gene enthalten und teils Gameten, die *D*-Gene enthalten. Bei der Verschmelzung der Gameten zu Zygoten werden drei derartige von verschiedener Art gebildet: Teils Zygoten, welche durch Zusammenschmelzung von *R*-Gameten entstehen und also zwei *R*-Gene enthalten und Homozygoten für *R*-Gene genannt werden; teils Zygoten, welche zwei *D*-Gene enthalten und Homozygoten für *D*-Gene genannt werden; schliesslich Zygoten, welche durch Zusammenschmelzung von *R*-Gameten und *D*-Gameten entstehen. Diese letzteren Zygoten werden Heterozygoten genannt. Im Hinblick auf die Eigenschaften, welche dieser Erbbeschaffenheit der Zygoten entsprechen, haben wir auch verschiedene Möglichkeiten. 1. Die eine Anlage kann über die andere *dominieren*. Dominiert das *D*-Gen, werden die Heterozygoten im Hinblick auf die Eigenschaft gleich den Homozygoten für das *D*-Gen. Man kann den entsprechenden Individuen nicht ansehen, ob sie eine verschiedene Erbbeschaffenheit haben; nur wenn man durch Kreuzungsexperimente oder ähnliches nachweisen kann, dass die Heterozygoten zwei verschiedene Arten von Gameten produzieren, kann man feststellen, dass sie in ihrer Beschaffenheit nicht mit den Homozygoten für das *D*-Gen identisch sind. Dominiert das *D*-Gen, so sagt man, dass das *R*-Gen *rezessiv* ist. In diesem Falle

erhält man also zwei verschiedene Arten von Eigenschaften. 2. Eine andere Möglichkeit besteht darin, dass die Heterozygoten eine Zwischenstellung zwischen den Homozygoten einnehmen. Sind die Homozygoten für die *R*-Gene weiss und die Homozygoten für die *D*-Gene schwarz, so werden die Heterozygoten in einem solchen Falle grau. Wir sprechen dann von einer *intermediären* Erbllichkeit. 3. Schliesslich besteht die Möglichkeit, dass die Heterozygoten eine von den Homozygoten völlig abweichende Beschaffenheit aufweisen. Wir können annehmen, dass die *R*-Homozygoten weiss sind und die *D*-Homozygoten schwarz. Erhalten in diesem Falle die Heterozygoten eine blaue Farbe, also eine von beiden Arten völlig abweichende Beschaffenheit, die auf der Seite der Variationsbreite liegt, von der man sich vorstellen kann, dass sie die Homozygoten verbindet, so sprechen wir von einer *extramediären* Erbllichkeit (dieser Ausdruck früher angewandt von GUNNAR DAHLBERG, 1942 a; JOHANSEN, 1926, spricht in diesem Falle von heterozygoten Konstruktionen, eine Bezeichnungsweise, die aber leicht zu Missverständnissen führen kann). Die Grenze zwischen intermediärer und extramediärer Erbllichkeit ist selbstverständlich nicht scharf.

### Geschlechtsgebundene monohybride Diallelie.

Bei dieser Form von Erbllichkeit haben die Männer nur eine der beiden Anlagen, die in Frage kommen können. Wir haben also zwei verschiedene Arten von Männern: Teils Männer mit einem *R*-Gen, welche in ihrer Eigenschaft mit den Homozygoten für das *R*-Gen übereinstimmen (die nur bei Frauen auftreten), teils Männer mit einem *D*-Gen, die in ihrer Eigenschaft mit Homozygoten für das *D*-Gen übereinstimmen (welche ebenfalls nur bei Frauen vorhanden sind). Wir haben also nur zwei verschiedene Arten von Männern sowohl im Hinblick auf die Anlagebeschaffenheit als auch im Hinblick auf die Eigenschaftsbeschaffenheit. Bei den Frauen hingegen treten die Gene in allelen Paaren auf und wir erhalten dann die verschiedenen Möglichkeiten der Anlage- und Eigenschaftsbeschaffenheit, über die wir weiter oben bei der monohybriden Diallelie berichtet haben. Eine Anlage, welche nur im *y*-Chromosom vorhanden ist, kann keine Wirkung haben, falls sie rezessiv ist. Ist sie dominant, so kommt die Eigenschaft nur bei Männern vor und wird nur auf die Söhne vererbt. Die Frauen sind keine Konduktoren.

Dieser theoretisch vorstellbare Erblchkeitsmechanismus wurde indessen nicht beim Menschen beobachtet.

### Monohybride Polyallelie.

Bei der monohybriden *Triallelie* sind drei verschiedene Arten von Genen vorhanden, von denen aber immer nur höchstens zwei gleichzeitig in einer Zygote vorkommen können. Die Eigenschaften, welche man untersucht, werden also durch ein Anlagepaar bedingt (Monohybridität) und durch Gene von dreierlei Art (*Triallelie*). Wir können annehmen, dass es sich um die Gene *D*, *R* und *I* handelt. Nachdem nur eines dieser Gene in einem Gameten vorkommen kann, erhalten wir entsprechend diesen verschiedenen Arten von Genen drei verschiedene Arten von Gameten. Nachdem nur zwei Gameten zu einer Zygote zusammenschmelzen können, erhalten wir drei verschiedene Arten von Homozygoten, nämlich *DD*, *RR* und *II*, sowie drei verschiedene Arten von Heterozygoten, nämlich *DR*, *RI* und *DI*. Im Hinblick auf die Eigenschaften dieser verschiedenen Arten von Individuen haben wir verschiedene Möglichkeiten. Die Homozygoten können für sich eine unterschiedliche Eigenschaft haben, die Heterozygoten können intermediär oder extramediär sein. Schliesslich kann es sich auch hier um Dominanz handeln. Die Möglichkeit liegt am nächsten, dass eine Anlage über die beiden anderen dominiert, also *D* über *R* und *D* über *I*, sowie dass die zweite Anlage über die dritte dominiert, z. B. *R* über *I*. In diesem Falle erhalten wir drei verschiedene Arten von Eigenschaftsträgern, nämlich *DD*-, *DR*- und *DI*-Individuen, die alle dieselbe Eigenschaft haben, *RR*- und *RI*-Individuen, die eine andere Eigenschaft haben, sowie schliesslich *II*-Individuen, die eine dritte Eigenschaft aufweisen. Eine andere Möglichkeit ist, dass zwei der Anlagen je für sich über eine dritte dominieren, dass aber die Heterozygoten mit diesen beiden dominanten Anlagen intermediäre oder extramediäre Beschaffenheit haben. In diesem letzteren Falle sagen wir, dass eine einfache Dominanz vorliegt, während wir im ersteren Falle, wo eine Anlage über die beiden übrigen dominiert, davon sprechen, dass eine doppelte Dominanz vorliegt.

Wir können uns selbstverständlich eine unbegrenzte Anzahl von Genen vorstellen, von welchen nur zwei gleichzeitig vorkommen. Alle Fälle, bei welchen mehr als zwei verschiedene Arten von Genen

vorkommen, fassen wir unter der Bezeichnung monohybride Polyallelie zusammen. Um uns die Möglichkeiten bei der monohybriden Polyallelie klar zu machen, nehmen wir an, dass es sich um eine willkürliche Anzahl verschiedener Gene, die wir  $n$  nennen, handelt. In diesem Falle können wir  $n$  verschiedene Arten von Gameten erhalten. Mit Hilfe von Regeln aus der Kombinationslehre können wir berechnen, wie viele Arten von Zygoten durch Kombination dieser verschiedenen Arten von Gameten gebildet werden können.

	$G_1$	$G_2$	$G_3$	$G_4$	$G_5$
$G_1$	$G_1 G_1$	$G_1 G_2$	$G_1 G_3$	$G_1 G_4$	$G_1 G_5$
$G_2$	$G_2 G_1$	$G_2 G_2$	$G_2 G_3$	$G_2 G_4$	$G_2 G_5$
$G_3$	$G_3 G_1$	$G_3 G_2$	$G_3 G_3$	$G_3 G_4$	$G_3 G_5$
$G_4$	$G_4 G_1$	$G_4 G_2$	$G_4 G_3$	$G_4 G_4$	$G_4 G_5$
$G_5$	$G_5 G_1$	$G_5 G_2$	$G_5 G_3$	$G_5 G_4$	$G_5 G_5$

Abb. 1. Vgl. Text.

In diesem Falle handelt es sich um eine Serie von Elementen (Gameten) von  $n$  verschiedenen Arten. Aus dieser Serie wollen wir zwei Elemente herausnehmen und sie miteinander zu einem Paar kombinieren; die Frage, welche sich dann ergibt, ist: Wie viele verschiedene Arten von Paaren, von Zweiergruppen, können wir bilden? In erster Linie können wir nun jeden Gameten mit einer bestimmten Art von Gen mit sich selbst kombinieren. Wir erhalten auf diese Weise Homozygoten und diese weisen ebensoviele verschiedene Arten auf wie die Gameten, also  $n$  verschiedene Arten. Die Heterozygoten erhalten wir dadurch, dass wir aus den verschiedenen Elementen zwei Gruppen bilden, wobei jedoch die Ordnung zwischen den Elementen nicht berücksichtigt wird, da es ja ohne Bedeutung ist, ob man Gen Nr. 1 mit Gen Nr. 2 oder Gen Nr. 2 mit Gen Nr. 1 kombiniert. In diesem Falle erhalten wir also gemäss den Regeln, auf welche wir oben hingewiesen haben,  $\frac{n(n-1)}{1 \cdot 2}$  verschiedene Arten von Heterozygoten. Insgesamt erhalten wir also bei monohybrider Polyallelie, wenn es sich um  $n$  verschiedene Gene und folglich um  $n$  verschiedene Gameten handelt,  $n + \frac{n(n-1)}{2}$  ihrer erblichen Be-

schaffenheit nach verschiedene Individuen. Der Prozess wird in Abb. 1 graphisch dargestellt. Längs zweier anstossenden Seiten eines Rechtecks werden die verschiedenen Arten von Gameten (Genen), die in Frage kommen können, aufgeschrieben. Wir bezeichnen die Gameten mit  $G_1$ ,  $G_2$ ,  $G_3$ ,  $G_4$  und  $G_5$ , so dass das Schema also die Zygotenbildung bei monohybrider Quintallelie umfasst. Dadurch, dass in jedes Diagonalfeld von links oben nach rechts unten die Bezeichnungen eingeführt werden, die in den zum Feld gehörigen Reihen an den Seiten des Rechtecks stehen, werden die Homozygoten erhalten. Die Heterozygoten werden durch Ausführung der entsprechenden Operation für die Felder, die oberhalb (oder unterhalb) der diagonal belegenen Felder liegen, erhalten. Die Felder unterhalb der diagonal belegenen enthalten dann dieselben Bezeichnungen (wenn auch in umgekehrter Ordnung) wie die Felder oberhalb.

Im Hinblick auf die Beschaffenheit dieser Individuen haben wir mit verschiedenen Kombinationen von einfacher oder mehrfacher Dominanz und intermediärem oder extramediärem Vererbungstypus zu rechnen.

### Geschlechtsgebundene monohybride Polyallelie.

Bei dieser Form von Vererbung sind für die Frauen dieselben Verhältnisse vorhanden, wie sie vorher für die monohybride Polyallelie beschrieben wurden. Die Männer haben hingegen nur ein Gen von den verschiedenen Arten von Genen, um die es sich hier handelt. Ist diese Anzahl  $n$ , so haben wir also bei den Männern ebenfalls  $n$  Arten erblich unterschiedlicher Individuen und diese haben auch alle verschiedene Eigenschaften. Hat man also bei der geschlechtsgebundenen monohybriden Polyallelie (oder Diallelie) mehrere verschiedene Arten von Eigenschaften bei den Frauen als bei den Männern, so kann man somit davon ausgehen, dass die Eigenschaftsträger bei den Frauen, denen die entsprechenden bei den Männern fehlen, Heterozygoten sind.

### Polyhybride Diallelie.

Bei der dihybriden Diallelie sind zwei Anlagepaare und eine Verschiedenheit zwischen den beiden Genen in jedem der Paare vorhanden. Wir nehmen an, dass das eine Paar aus den Genen  $D_1$

und  $R_1$ , das zweite Paar aus den Genen  $D_2$  und  $R_2$  besteht. Bei der Bildung von Gameten kommt nur das eine der Gene in jedem Paar mit in einen Gameten. Wir kombinieren also das  $D_1$ -Gen mit jedem der beiden Gene im zweiten Genpaar und erhalten Gameten von der Beschaffenheit  $D_1 D_2$  und  $D_1 R_2$ . Wir kombinieren dann das  $R_1$ -Gen mit jedem der beiden Gene in dem zweiten Genpaar und erhalten dann die Gameten  $R_1 D_2$  und  $R_1 R_2$ . Wir erhalten also vier verschiedene Arten von Gameten und die Gametenarten können formal durch die folgende Multiplikation berechnet werden:

$$(D_1 + R_1) (D_2 + R_2) = D_1 D_2 + D_1 R_2 + R_1 D_2 + R_1 R_2$$

	$D_1$	$R_1$
$D_2$	$D_1 D_2$	$R_1 D_2$
$R_2$	$D_1 R_2$	$R_1 R_2$

Abb. 2. Vgl. Text.

Der Prozess wird durch Abb. 2 graphisch veranschaulicht. Entlang beiden Seiten eines Rechtecks werden die entsprechenden Genpaare aufgeschrieben: das eine Paar entlang der horizontalen Seite, das andere Paar entlang der vertikalen. Das Rechteck wird in vier Felder eingeteilt und in den Feldern wird die Zusammensetzung der Gameten erhalten, wenn man die Bezeichnungen einschreibt, die vertikal und horizontal an den Seiten des Rechtecks über jedem einzelnen Feld stehen.

Wenn die Gameten sich mit einander zu Zygoten vereinen, kann man die entsprechende Überlegung und Formel, die wir für die monohybride Polyallelie angewandt haben, benutzen. In diesem Falle handelt es sich um vier verschiedene Gametenarten, vier verschiedene Arten von Elementen, die sich zufällig miteinander zu einem Paar vereinen. So können sich erstens gleiche Elemente miteinander vereinen; nachdem wir vier verschiedene Arten von Elementen haben, erhalten wir also vier verschiedene Arten von Homozygoten. Die Heterozygoten werden nach der Formel  $\frac{n(n-1)}{2}$  erhalten. In diesem Falle ist  $n = 4$ . Wir erhalten also sechs verschiedene Arten von Heterozygoten. Der Prozess wird graphisch auf die entsprechende Weise wie bei der monohybriden Polyallelie veranschaulicht.

Entlang den Seiten eines Rechtecks (Abb. 3) schreiben wir die verschiedenen Gametenarten. Das Rechteck wird in Felder eingeteilt. Durch Einschreiben der beiden Gametenkonstruktionen, die horizontal vor den *diagonalen* Feldern und vertikal über ihnen stehen, werden die Homozygoten erhalten. Dadurch, dass auf entsprechende Weise die Felder *oberhalb* (oder *unterhalb*) dieser diagonalen Felder geschrieben werden, werden die Heterozygoten erhalten. Es ist ja deutlich, dass die Felder oberhalb und unterhalb der Diagonale dieselbe Beschaffenheit erhalten.

	$D_1 D_2$	$D_1 R_2$	$R_1 D_2$	$R_1 R_2$
$D_1 D_2$	$D_1 D_1 D_2 D_2$	$D_1 D_1 D_2 R_2$	$D_1 R_1 D_2 D_2$	$D_1 R_1 D_2 R_2$
$D_1 R_2$	$D_1 D_1 D_2 R_2$	$D_1 D_1 R_2 R_2$	$D_1 R_1 D_2 R_2$	$D_1 R_1 R_2 R_2$
$R_1 D_2$	$D_1 R_1 D_2 D_2$	$D_1 R_1 D_2 R_2$	$R_1 R_1 D_2 D_2$	$R_1 R_1 D_2 R_2$
$R_1 R_2$	$D_1 R_1 D_2 R_2$	$D_1 R_1 R_2 R_2$	$R_1 R_1 D_2 R_2$	$R_1 R_1 R_2 R_2$

Abb. 3. Vgl. Text.

Mathematisch erhält man die verschiedenen Möglichkeiten für die Zygoten durch Multiplikation, gemäss folgender Formel:

$$(D_1 D_2 + D_1 R_2 + R_1 D_2 + R_1 R_2) (D_1 D_2 + D_1 R_2 + R_1 D_2 + R_1 R_2)$$

Die Koeffizienten besitzen in diesem Zusammenhange kein Interesse und wir erhalten also in diesem Falle 4 verschiedene Arten von Homozygoten und 6 verschiedene Arten von Heterozygoten, insgesamt also 10 verschiedene Arten.

Man kann sich selbstverständlich vorstellen, dass es sich hier um eine durchgehende intermediäre oder durchgehende extramediäre Vererbung handelt. Wir erhalten in diesem Falle auch 10 verschiedene Arten von Eigenschaftsträgern. Eine andere Möglichkeit stellt eine einfache Dominanz dar, in diesem Falle dominiert also die eine Anlage über die zweite in jedem Paar, während hingegen keine Anlage des einen Paares über eine im zweiten Paar dominiert. In diesem Falle erhalten wir vier verschiedene Arten von Eigenschaften. Schliesslich hat man auch mit der Möglichkeit zu rechnen, dass die eine dominante Anlage über die zweite dominiert. Wir erhalten dann nur drei verschiedene Arten von Eigenschaftsträgern.



Wir wollen schliesslich wissen, wie das Ergebnis ausfällt, wenn es sich um eine grössere Anzahl von Genpaaren handelt, die sich miteinander kombinieren. Wir nehmen an, dass es sich um  $m$  Paare von allelen Genen handelt und wir haben also in diesem Falle  $2m$  verschiedene Arten von Genen. Wir nehmen also an, dass die verschiedenen Genpaare folgende Beschaffenheit haben:

$$D_1 R_1; D_2 R_2; D_3 R_3 \dots D_m R_m.$$

Bei der Bildung von Gameten verhält es sich so, dass ein Gen eines jeden Paares sich mit einem Gen aus allen übrigen Paaren kombiniert. Mathematisch erhält man die Gametenart aus dem Ausdruck:

$$(D_1 + R_1) \cdot (D_2 + R_2) \cdot (D_3 + R_3) \dots (D_m + R_m)$$

Die Anzahl der verschiedenen Arten von Gameten wird also  $2^m$ .

Kombinieren sich die Gameten miteinander, so haben wir in erster Linie mit der Möglichkeit zu rechnen, dass Gameten derselben Art sich miteinander zu Homozygoten vereinen. Wir haben  $2^m$  verschiedene Arten von Gameten und erhalten also  $2^m$  verschiedene Arten von Homozygoten. Bei der Bildung der Heterozygoten kombinieren wir wie gewöhnlich  $2^m$  Gameten, ohne Hinsicht auf die Reihenfolge der Gameten in den erhaltenen Paaren, miteinander.

Wir erhalten also  $\frac{2^m (2^m - 1)}{2}$ .

Selbstverständlich liegt auch in dieser Situation im Hinblick auf die Eigenschaftsbeschaffenheit dieser Individuen eine grosse Anzahl von Möglichkeiten von Kombinationen zwischen den verschiedenen Arten von dominanter und intermediärer oder extramediärer Vererbung vor.

### Geschlechtsgebundene polyhybride Diallelie.

Bei der geschlechtsgebundenen polyhybriden Diallelie haben wir wie gewöhnlich eben so viele erblich verschiedene Arten von Individuen und verschiedene Arten von Eigenschaftsträgern, wie wir Gameten haben, also in diesem Falle, mit den vorher angewandten Bezeichnungen,  $2^m$ , ihrer Vererbung und Eigenschaftsbeschaffenheit nach verschiedene Männer. Für Frauen gilt das, was vorher über polyhybride Diallelie gesagt wurde. Handelt es sich um inter- oder

extramediäre Vererbung, erhalten wir also mehr verschiedene Arten von Eigenschaftsträgern bei den Frauen als bei den Männern.

### Polyhybride Polyallelie.

Theoretisch ist selbstverständlich auch die Möglichkeit vorhanden, dass wir eine willkürliche Anzahl von allelen Paaren haben und dass für ein oder mehrere dieser Paare eine willkürliche Anzahl von Genen vorhanden ist, von welchen aber nur zwei gleichzeitig vorkommen können. Es ist klar, dass diese Situation äusserst kompliziert ist. Dies um so mehr, als bei der Entstehung von Eigenschaften auch die Möglichkeit vorliegt, dass man mit verschiedenen Arten von Dominanz, intermediärer und extramediärer Vererbung rechnen muss. Es dürfte kaum Interesse haben zu versuchen die hierbei vorliegenden Möglichkeiten näher aufzuklären. Der Vollständigkeit halber wollten wir nur dieses Verhalten erwähnen.

## Die Zusammensetzung der Population bei Panmixie.

Will man die Erbliehkeitsverhältnisse in menschlichen Populationen untersuchen, so geht man in erster Linie von der Annahme aus, dass die Ehen in einer Population zufallsbedingt geschlossen werden und dass sich die verschiedenen Gruppen von Eigenschaftsträgern in gleichem Ausmasse fortpflanzen. In einer Population, in welcher dies der Fall ist, spricht man von dem Vorhandensein einer Panmixie. Diese Voraussetzung gilt sicher mit zufriedenstellender Genauigkeit in vielen Fällen.

Panmixie liegt selbstverständlich nicht immer vor. Abweichungen von den Ergebnissen, die man bei zufallsbedingten Kreuzungen, Panmixie, erhalten würde, können nur auf fünf Arten erhalten werden (vgl. DAHLBERG 1939 b):

1. Eine Ursache für Abweichungen von der Zusammensetzung bei Panmixie ist die *Selektion*. Die im Hinblick auf die Vererbung verschiedenen Arten von Individuen in einer Bevölkerung können eine unterschiedliche Fruchtbarkeit haben. Dies führt zu fortschreitenden Verschiebungen in der Zusammensetzung der Bevölkerung von Generation zu Generation; da in Wirklichkeit die Generationen ineinander übergreifen und nicht scharf getrennt sind, führt dies zu einer von der Panmixie kontinuierlich immer mehr abweichenden Zusammensetzung der Bevölkerung.

2. *Verwandtschaftsehe* bedingt eine erhöhte Wahrscheinlichkeit für Homozygotie. In der Annahme, dass eine Panmixie vorliegt, ist inbegriffen, dass auch Verwandte zufallsbedingt miteinander die Ehe schliessen. Der Grad von Homozygotie, der hierdurch verursacht wird, ist also in den Ergebnissen enthalten, die unter der Voraussetzung berechnet werden, dass eine Panmixie vorliegt. Würden Verwandtschaftsehen indessen in einem grösseren oder geringeren Ausmasse als bei Panmixie erwartet vorkommen, so wird hierdurch

selbstverständlich eine abweichende Zusammensetzung der Population verursacht.

3. Verheiraten sich die Individuen zufällig mit einander, so geschieht dies doch immer nur innerhalb eines bestimmten begrenzten Gebietes unter einer gewissen Anzahl von Individuen. Wird eine Ehe in einem Volk geschlossen, so ist es ja klar, dass ein Individuum aus einer bestimmten sozialen Klasse und einem bestimmten geographischen Gebiet nur die Möglichkeit hat, sich mit einer begrenzteren Anzahl von Individuen in der Population, die das Land bevölkert, zu verheiraten. Eine grössere Population zerfällt aus diesem Gesichtspunkte in Teilpopulationen, die wir *Isolate* nennen. Diese werden durch Isolatgrenzen von geographischer und sozialer Art voneinander getrennt. Innerhalb dieser Isolate kann man voraussetzen, dass eine Panmixie stattfindet. Haben die Isolate voneinander abweichende Beschaffenheit, so erhält man bei dem Zusammenschlagen der Isolate zu einer Population eine andere Zusammensetzung des Gesamtmaterials als man erhalten würde, wenn eine Panmixie in der ganzen Bevölkerung stattgefunden hätte.

4. »*Assortative mating*» (Gattenwahl); dieser Ausdruck bedeutet, dass die Eigenschaftsträger in grösserem Ausmasse als durch ein zufallsbedingtes Zusammentreffen sich miteinander verheiraten, was Abweichungen von der Panmixie bedeuten kann. Der Prozess führt zu einer Zunahme der Homozygotenfrequenz in einer Bevölkerung.

5. Durch *Mutationen* kann, prinzipiell gesehen, die Erbbeschaffenheit in einer Population von Generation zu Generation verändert werden, so dass die Bevölkerung eine Zusammensetzung erhält, die von der abweicht, die man bei Panmixie erwartet.

Unter diese Hauptrubriken fallen Varianten der verschiedenen Mechanismen für die Abweichungen von Panmixie. Prinzipiell gesehen dürfte man aber jede Abweichung auf eine dieser Rubriken zurückführen können.

Man kann sicher behaupten, dass man in vielen Fällen berechtigt ist anzunehmen, dass eine Panmixie vorliegt. In diesem Falle besitzt die Bevölkerung eine konstante Zusammensetzung und der Jengehalt verändert sich nicht von Generation zu Generation. Die verschiedenen Gene pflanzen sich ja in gleichem Ausmasse weiter fort. Wenn man indessen in einem gegebenen Fall Formeln anwenden soll, die auf die Voraussetzung aufgebaut wurden, dass eine

Panmixie vorliegt, so muss man selbstverständlich versuchen, sich eine Vorstellung darüber zu bilden, dass diese Voraussetzung wirklich mit zufriedenstellender Genauigkeit gültig ist.

### Die Zusammensetzung der Population bei monohybrider Diallelie.

Wollen wir die Zusammensetzung der Population für eine monohybride diallele Eigenschaft bei Panmixie berechnen, so ist es am geeignetsten von der Beschaffenheit der Gameten in der Population auszugehen. Wir nehmen an, dass unter den Gameten, die von den Individuen in der Population produziert werden, die rezessive Anlage  $R$  in einer Frequenz von  $r$  und die dominante Anlage  $D$  in einer Frequenz von  $d$  vorhanden ist. Die Wahrscheinlichkeit dafür, dass man bei einer zufälligen Auswahl einen Gameten mit dem rezessiven Gen erhält, ist also  $r$ . Die Wahrscheinlichkeit, dass man einen Gameten mit dem dominanten Gen erhält, ist  $d$ ; nachdem diese beiden Wahrscheinlichkeiten einander ausschliessen, ist ihre Summe 1.

Also

$$r + d = 1$$

und

$$r = 1 - d$$

Wenn nun die Individuen, welche diese Gameten produzieren, sich miteinander verheiraten, so bedeutet dies eine Einschränkung der Bewegungsfreiheit der Gameten. In einer bestimmten Ehe können ja Zygoten ausschliesslich durch Verbindungen zwischen denjenigen Gametenarten, welche die Kontrahenten produzieren, entstehen. Die Monogamie bedingt also eine gewisse Einschränkung der zufallsbedingten Bewegungsfreiheit der Gameten. Diese Einschränkung besitzt indessen in dem vorliegenden Falle keine Bedeutung. Man kann somit von der Annahme ausgehen, als ob alle Gameten, welche die Individuen einer Population produzieren, eine einzige Masse bilden und in einem See gesammelt würden, in welchem die Gameten zufallsbedingt zusammentreffen würden. In der Tat handelt es sich um einen komplizierteren Mechanismus, dieser ergibt aber dasselbe Ergebnis, wie der oben beschriebene einfache Mischungsprozess. Wir gehen also davon aus, dass die Gameten zufälligerweise zusammentreffen und die Wahrscheinlichkeit der Kom-

binationen, die entstehen können, kann leicht berechnet werden, nachdem wir die Wahrscheinlichkeit für das Vorliegen der verschiedenen Arten von Gameten kennen.

Ist die Wahrscheinlichkeit für einen  $R$ -Gameten  $r$ , so besteht für einen  $R$ -Gameten die Wahrscheinlichkeit  $r \cdot r = r^2$  für ein Zusammentreffen mit einem anderen  $R$ -Gameten zu einer Zygote mit der Beschaffenheit  $RR$ .

Ist die Wahrscheinlichkeit für einen  $D$ -Gameten  $d$ , so besteht für einen  $D$ -Gameten die Wahrscheinlichkeit  $d \cdot d = d^2$  für ein Zusammentreffen mit einem anderen  $D$ -Gameten zu einem Zygoten mit der Beschaffenheit  $DD$ .

Die Wahrscheinlichkeit für das Zusammentreffen eines  $R$ -Gameten und eines  $D$ -Gameten zu einem Zygoten mit der Beschaffenheit  $RD$  (oder  $DR$ ) ist schliesslich  $2rd$ .

Das Ergebnis ist also:

Die Wahrscheinlichkeit für rezessive Homozygoten ( $RR$ ), d. h. für rezessive Eigenschaftsträger, beträgt  $\dots r^2$ .

Die Wahrscheinlichkeit für Heterozygoten ( $RD$ ) ist  $\dots 2rd$ .

Die Wahrscheinlichkeit für dominante Homozygoten ( $DD$ ) beträgt  $\dots d^2$ .

Die Wahrscheinlichkeit für dominante Eigenschaftsträger ( $RD + DD$ ) beträgt also  $= 2rd + d^2 = 1 - r^2$  und ferner weil  $r + d = 1$  ist

$$r^2 + 2rd + d^2 = 1. \quad (1)$$

Da wir wissen, dass die Homozygoten ausschliesslich  $R$ - bzw.  $D$ -Gameten produzieren, während die Heterozygoten zur Hälfte  $R$ - und zur Hälfte  $D$ -Gameten produzieren, muss unter der Voraussetzung, dass die Individuen sich gleich stark vermehren, die Menge an  $R$ -Gameten unter den Gameten, welche sie erzeugen, aus der Anzahl rezessiver Homozygoten + der halben Anzahl von Heterozygoten zu erhalten sein. Dasselbe gilt auch für die  $D$ -Gameten.

Also muss

$$r = r^2 + rd$$

und

$$d = d^2 + rd$$

sein.

Kennt man somit die Anzahl rezessiver Eigenschaftsträger in einer Population von gewisser Grösse (und folglich auch die Anzahl

der dominanten Eigenschaftsträger), so kann man  $r$  und  $d$  mit Hilfe der oben stehenden Formeln leicht berechnen. Mit Hilfe der für  $r$  und  $d$  erhaltenen Werte kann man hernach die Anzahl der Heterozygoten in der Bevölkerung berechnen.<sup>1</sup>

Um die Beschaffenheit einer Bevölkerung, welche gemäss dieser Formeln zusammengesetzt ist, zu veranschaulichen, sei auf Abb. 4 verwiesen. In dieser Abbildung ist die Seite des Quadrates im Ver-

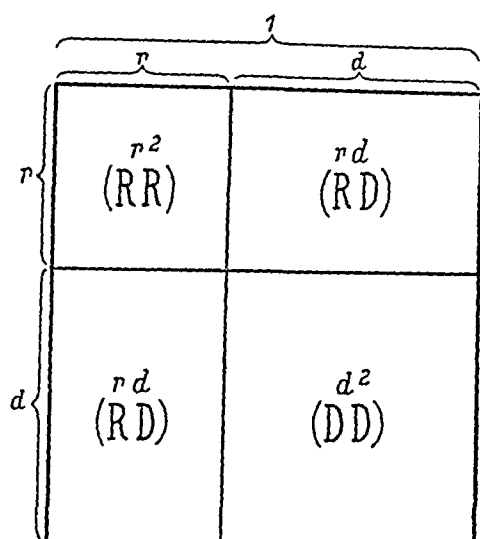


Abb. 4. Verteilung der Zygotentypen in einer Population (RR, RD und DD) mit der Proportion der Gene  $r : d = 2 : 3$ . (Nach DAHLBERG-HULTERANTZ 1927.)

hältnis  $r : d$  aufgeteilt (im vorliegenden Falle  $2 : 3$ ). Die Grösse des Feldes  $r^2$  und der beiden Felder  $rd$  und des Feldes  $d^2$  veranschaulicht die Frequenz der verschiedenen Arten von Individuen (RR-, RD- und DD-Individuen), welche in einer Bevölkerung mit einem derartigen Gehalt an Genen entstehen. Das Feld  $r^2$  entspricht also den rezessiven Eigenschaftsträgern und die übrigen Felder zusammen entsprechen den dominanten Eigenschaftsträgern.

Aus dem Diagramm in Abb. 5 kann die Zusammensetzung, welche man bei Panmixie, bei verschiedenem Gengehalt in der Bevölkerung, erhält, entnommen werden. Die gestrichelt gezeichnete Diagonale gibt die verschiedenen möglichen Proportionen (von  $\frac{0}{1}$  bis  $\frac{1}{0}$ ;  $r$

<sup>1</sup> Der erste, der Formeln für die Zusammensetzung der Population bei Panmixie angegeben und gezeigt hat, dass die Erbbeschaffenheit der Population bei Panmixie sich konstant hält, war HARDY (1908). Bereits früher hat PEARSON (1903) dasselbe für die Proportionen  $1 : 2 : 1$  aufgezeigt.





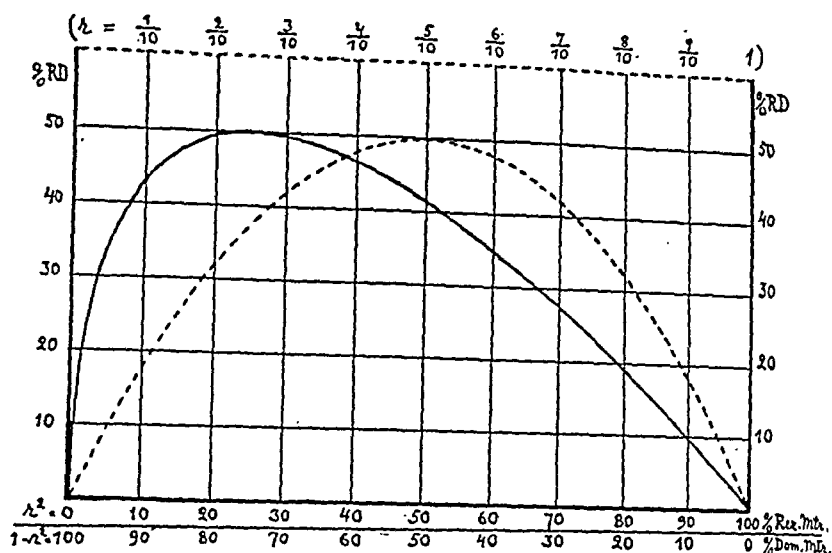


Abb. 6. Prozentwert der Heterozygoten bei verschiedener Proportion der rezessiven und dominanten Eigenschaftsträger (ausgezogene Linie; unten stehende Zahlen) und bei verschiedenem Gehalt an rezessiven Genen (gestrichelte Linie; oben stehende Zahlen). (Nach DAHLBERG-HULTERANTZ 1927.)

halt an  $R$ -Genen ( $r$ ), welcher in der Abb. angegeben ist. In Tabelle 1 werden exakte zahlenmässige Werte für einige verschiedene Möglichkeiten der Zusammensetzung einer Bevölkerung bei monohybrider Diallelie angeführt.

Aus den Diagrammen und der Tabelle geht hervor, dass die Heterozygoten höchstens 50 % einer Bevölkerung ausmachen können und dass dieses Maximum dann erreicht wird, wenn  $R$ - und  $D$ -Gene gleich häufig vorkommen, d. h. beide die Wahrscheinlichkeit von  $\frac{1}{2}$  besitzen; die rezessiven Homozygoten machen in diesem Falle also  $\frac{1}{4}$  der Bevölkerung aus. In diesem Falle wird die Zusammensetzung der Bevölkerung durch die klassischen Werte von MENDEL  $\frac{1}{4} : \frac{1}{2} : \frac{1}{4}$  charakterisiert. Wenn  $r = \frac{1}{3}$ , sind übrigens die Hälfte der dominanten Eigenschaftsträger Heterozygoten, und wenn  $r = \frac{2}{3}$ , finden sich gleich viele rezessive Eigenschaftsträger wie Heterozygoten. Je grösser der Unterschied zwischen der Frequenz der  $R$ - und  $D$ -Gene wird, desto geringer fällt die Frequenz der Heterozygoten, verglichen mit der Bevölkerung in ihrer Gesamtheit, aus.

Die Formeln, die wir oben aufgestellt haben, besitzen also unter anderem auch deswegen Interesse, weil sie eine Berechnung der Anzahl der Heterozygoten bei monohybrider dialleler rezessiv-dominanter Vererbung (und selbstverständlich auch bei inter- oder extra-

TABELLE 1.

Prozentzahl der Heterozygoten bei verschiedener Genproportion.

RR-Zygoten (Rez. Etr.) 100 $r^2$	RD-Zygoten 100 $2rd$	DD-Zygoten 100 $d^2$	RR-Zygoten (Rez. Etr.) 100 $r^2$	RD-Zygoten 100 $2rd$	DD-Zygoten 100 $d^2$
0.01	1.08	98.01	45	44.16	10.84
0.05	4.37	95.63	46	43.65	10.35
0.1	6.12	93.78	47	43.11	9.89
0.2	8.54	91.26	48	42.56	9.44
0.3	10.35	89.35	49	42	9
0.4	11.85	87.75	50	41.42	8.58
0.5	13.14	86.36	51	40.83	8.17
0.6	14.29	85.11	52	40.22	7.78
0.7	15.33	83.97	53	39.60	7.40
0.8	16.29	82.91	54	38.97	7.03
0.9	17.17	81.93	55	38.32	6.68
			56	37.67	6.33
1	18	81	57	37	6
2	24.28	73.72	58	36.32	5.68
3	28.64	68.36	59	35.62	5.38
4	32	64	60	34.92	5.08
5	34.72	60.28	61	34.20	4.80
6	36.99	57.01	62	33.48	4.52
7	38.92	54.09	63	32.75	4.25
8	40.57	51.43	64	32	4
9	42	49	65	31.25	3.75
10	43.25	46.75	66	30.48	3.52
11	44.33	44.67	67	29.71	3.29
12	45.28	42.72	68	28.92	3.08
13	46.11	40.89	69	28.13	2.87
14	46.83	39.17	70	27.33	2.67
15	47.46	37.54	71	26.52	2.48
16	48	36	72	25.71	2.29
17	48.46	34.54	73	24.88	2.12
18	48.85	33.15	74	24.05	1.95
19	49.18	31.82	75	23.21	1.79
20	49.44	30.56	76	22.36	1.64
21	49.65	29.35	77	21.5	1.5
22	49.81	28.19	78	20.64	1.36
23	49.92	27.08	79	19.76	1.24
24	49.98	26.02	80	18.89	1.11
25	50	25	81	18	1
26	49.98	24.02	82	17.11	0.89
27	49.92	23.03	83	16.21	0.79
28	49.83	22.17	84	15.30	0.70
29	49.70	21.30	85	14.39	0.61
30	49.54	20.46	86	13.47	0.53
31	49.36	19.64	87	12.55	0.45
32	49.14	18.86	88	11.62	0.38
33	48.89	18.11	89	10.68	0.32
34	48.62	17.38	90	9.74	0.26
35	48.32	16.68	91	8.79	0.21
36	48	16	92	7.83	0.17
37	47.66	15.34	93	6.87	0.13
38	47.29	14.71	94	5.91	0.09
39	46.90	14.10	95	4.94	0.06
40	46.49	13.51	96	3.96	0.04
41	46.06	12.94	97	2.98	0.02
42	45.61	12.39	98	1.99	0.01
43	45.15	11.85	99	0.9975	0.0025
44	44.66	11.34	100	0	0

mediärer Vererbung) ermöglichen. Da bei dieser Form von Vererbung die Heterozygoten im Aussehen mit den dominanten Homozygoten übereinstimmen, haben wir ja keine Möglichkeit die Heterozygoten direkt zu beobachten und zu bestimmen.

Es ist nun von besonderem Interesse zu untersuchen, wie sich der Gehalt an Heterozygoten zu dem Gehalt an Homozygoten von rezessiver bzw. dominanter Art verhält. Mit Hilfe der Gleichungen, die wir oben aufgestellt haben, ergibt sich, dass

$$\frac{\text{Heterozygoten}}{\text{Rezessive Eigenschaftsträger}} = \frac{2rd}{r^2} = \frac{2(1-r)}{r} \quad (2)$$

$$\frac{\text{Heterozygoten}}{\text{Dominante Eigenschaftsträger}} = \frac{2rd}{d^2 + 2rd} = \frac{2r}{1+r} \quad (3)$$

Betrachten wir nun zuerst die Gleichung für das Verhalten zwischen Heterozygoten und rezessiven Eigenschaftsträgern, so finden wir, dass falls  $r$  zahlenmässig klein ist, sich 0 nähert, so nähert sich das Verhältnis zwischen beiden  $\infty$ . Ist  $r$  zahlenmässig klein, so wird die Frequenz der rezessiven Eigenschaftsträger ( $r^2$ ) äusserst klein. Sind also die rezessiven Eigenschaftsträger in einer Bevölkerung sehr selten, so finden sich verhältnismässig viele Heterozygoten oder latente Eigenschaftsträger in der Bevölkerung. Gesehen vom Standpunkt der Bevölkerung aus, sind also sowohl die latenten als auch die manifesten Eigenschaftsträger selten, die manifesten Eigenschaftsträger aber doch unverhältnismässig seltener als die latenten.

Dies gilt auch für das Verhältnis der Heterozygoten zu den dominanten Homozygoten. Handelt es sich um eine seltene Eigenschaft, so besteht der Hauptteil der Eigenschaftsträger aus Heterozygoten. Umgekehrt, wenn es sich um eine sehr häufig vorkommende dominante Anlage handelt (nahe 1) stellen die Heterozygoten einen verschwindenden Bruchteil der dominanten Eigenschaftsträger dar.

Diese Schlüsse über das Verhältnis der Heterozygoten zu den Homozygoten besitzt für die Methodik der Erblichkeitsforschung eine gewisse Bedeutung. Geht man z. B. von Eigenschaftsträgern aus und untersucht deren Geschwister, so erhält man eine unterschiedliche Zusammensetzung des Geschwisterkreises bei verschiedener Frequenz einer Anlage. Theoretisch gesehen kann man aus der Beschaffenheit von Geschwisterkreisen die Frequenz der Anlage in der

Population berechnen, aus der die Geschwisterkreise genommen wurden, wenn man die Art der Vererbung kennt (vgl. DAHLBERG-HULTKRANTZ 1927). In der Praxis dürfte es aber kaum möglich sein auf diesem Wege zuverlässige Frequenzwerte zu erhalten. Theoretisch gesehen kann man ferner aus der Erbbeschaffenheit von Zwillingspaaren, die zweieiig sind, die Frequenz von Anlagen in der Population berechnen, aus der sie stammen. RIFE (1938) hat für solche Berechnungen Formeln aufgestellt, die auch in der Arbeit von DAHLBERG (1942 b) wiederzufinden sind. In der letztgenannten Arbeit wurden die Bezeichnungen angewandt, die auch in der vorliegenden Arbeit angewandt werden.

### Die Zusammensetzung der Population bei geschlechtsgebundener monohybrider Diallelie.

Bei der geschlechtsgebundenen monohybriden Diallelie wird die Eigenschaft, auf die man das Augenmerk richtet, bei den Männern durch eine einzige Anlage, bei den Frauen durch ein Anlagepaar, durch 2 Anlagen, bedingt. Die Männer bilden teils Geschlechtszellen, denen irgend eine Anlage völlig fehlt, die mit der Eigenschaft zu tun hat, und teils solche, welche eine Anlage enthalten und diese beiden Arten von Geschlechtszellen werden in gleich grossem Ausmasse gebildet, d. h. für beide besteht die Wahrscheinlichkeit  $\frac{1}{2}$ . Frauen bilden nur Geschlechtszellen, die eine Anlage haben. Bei der Befruchtung entstehen teils Individuen, die nur eine Anlage der betreffenden Eigenschaft aufweisen und die immer männliches Geschlecht erhalten, teils Individuen mit zwei Anlagen für die betreffende Eigenschaft, diese Individuen erhalten immer weibliches Geschlecht. Die Männer bilden also zwei Arten von Spermien und das Geschlecht eines Individuums wird durch die Beschaffenheit des Spermiums, das das Ei befruchtet, aus welchem das Individuum entstanden ist, bestimmt.

In mathematischer Hinsicht stellt die Zusammensetzung der Bevölkerung bei geschlechtsgebundener monohybrider Diallelie für Männer das einfachste Problem dar.

Wir nehmen, wie vorher, an, dass wir die Zusammensetzung der Bevölkerung unter Berücksichtigung einer rezessiven Anlage  $R$  mit der Frequenz  $r$  und einer dominanten Anlage  $D$  mit der Frequenz  $d$  berechnen wollen. Für die Frauen erhalten wir dann dieselben Ver-

hältnisse wie bei gewöhnlicher monohybrider Diallelie, d. h. wir erhalten rezessive Eigenschaftsträger oder  $RR$ -Individuen mit der Wahrscheinlichkeit  $r^2$  und dominante Heterozygoten mit der Wahrscheinlichkeit  $2rd$ , sowie dominante Homozygoten mit der Wahrscheinlichkeit  $d^2$ . Bezüglich der übrigen Verhältnisse verweisen wir auf das oben über monohybride Diallelie Gesagte.

Für die Männer liegen, wie erwähnt, die Verhältnisse noch einfacher. Die verschiedenen Arten von Eigenschaftsträgern erhalten dieselbe Frequenz wie die verschiedenen Genarten. Wir erhalten also Individuen mit der rezessiven Eigenschaft in  $r$  Fällen und Individuen mit der dominanten Eigenschaft in  $d$  Fällen. Da die beiden Anlagen niemals gleichzeitig bei einem Mann vorkommen können, kann nun die eine Anlage bei den Männern niemals über die zweite dominieren. Wir wenden die Bezeichnungen rezessive und dominante Eigenschaftsträger also für die Männer nicht ganz sinngemäss an, die Individuen aber, welche die  $R$ -Anlage haben und die wir also rezessive Eigenschaftsträger nennen, werden im Hinblick auf die Eigenschaft mit den rezessiven Eigenschaftsträgern unter den Frauen übereinstimmen.

Es ist nun von besonderem Interesse, dass falls man die Frequenz der rezessiven Eigenschaftsträger unter den Männern kennt, man durch Quadrierung dieses Wertes die Wahrscheinlichkeit der rezessiven Eigenschaftsträger unter den Frauen erhält, oder umgekehrt: falls man diese letztgenannte Wahrscheinlichkeit kennt, erhält man die Wahrscheinlichkeit der rezessiven Eigenschaftsträger bei Männern dadurch, dass man aus diesem Wert die Quadratwurzel zieht. — Die Beziehung zwischen rezessiven Eigenschaftsträgern bei Männern und unter den Frauen ist ja gemäss unseren Formeln

$$\frac{\text{»rezess.» Eigenschaftstr. unter Männern}}{\text{rezess. Eigenschaftstr. unter Frauen}} = \frac{r}{r^2} = \frac{1}{r}$$

Wir können das Gesagte durch ein Beispiel beleuchten. Bei einer Untersuchung von Knaben und Mädchen auf Farbenblindheit wurden folgende Werte erhalten (WAALER 1927). Unter den Knaben Protanope 1 %, Protanomale 1 %, Deuteranope 1 %, Deuteranomale 5 %, insgesamt also rund 8 %. Unter den Mädchen würde man dann 0.01 % Protanope, 0.01 % Protanomale, 0.01 % Deuteranope und 0.25 % Deuteranomale, d. h. insgesamt 0.28 % erwarten. Die theoretischen Werte wurden nach den angegebenen Formeln derart er-

halten, dass die Frequenzwerte der Knaben quadriert worden sind. Der beobachtete Wert bei Mädchen beträgt  $0.44 \pm 0.07\%$ , der erwartete und der beobachtete Wert stimmen somit innerhalb des dreifachen mittleren Fehlers miteinander überein. (Man muss auch berücksichtigen, dass die beobachteten Werte bei den Knaben einen mittleren Fehler aufweisen, der die berechneten Werte beeinflusst.)

Die angegebene Formel besitzt auch aus einem anderen Gesichtspunkt Interesse. Die Relation zwischen männlichen und weiblichen rezessiven Eigenschaftsträgern ist  $1:r$ . Ist nun  $r$  eine sehr kleine

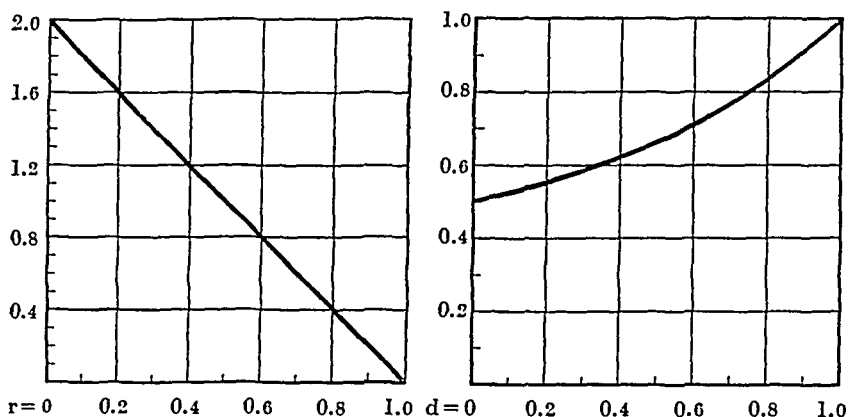


Abb. 7 und 8. Vgl. Text.

Zahl, d. h. die männlichen Eigenschaftsträger sind in der in Frage kommenden Population äusserst selten, so werden aber die männlichen Eigenschaftsträger doch unendlich viel häufiger vorkommen als die weiblichen und letztere infolge dessen viel seltener als die männlichen. Nehmen wir an, dass z. B. die männlichen Eigenschaftsträger bei 1:1000 Individuen vorhanden sind, so werden die weiblichen Eigenschaftsträger bei 1:1 000 000 vorkommen.

Eine andere Relation, die auch Interesse besitzt, ist das Verhältnis zwischen weiblichen, latenten Eigenschaftsträgern und männlichen Eigenschaftsträgern. Dieses Verhältnis wird durch  $2rd:r$  und vereinfacht  $2(1-r):1$  ausgedrückt. Aus diesem Ausdruck ergibt sich, dass falls die männlichen Eigenschaftsträger selten sind ( $r$  nähert sich 0), so werden die latenten weiblichen Eigenschaftsträger an Zahl doppelt so viel sein wie die männlichen Eigenschaftsträger. Handelt es sich um eine häufiger vorkommende Eigenschaft, so ist das Übergewicht geringer. Ist die rezessive Eigenschaft bei der

Hälfte der männlichen Bevölkerung vorhanden, so machen die weiblichen Eigenschaftsträger eine gleich grosse Anzahl aus. Ist die betreffende Eigenschaft noch häufiger vorkommend, so sinkt die relative Anzahl der Eigenschaftsträger unter den Frauen noch mehr. Die Relation wird durch das vorstehende Diagramm veranschaulicht (Abb. 7).

Das Verhältnis zwischen männlichen dominanten Eigenschaftsträgern und weiblichen dominanten Eigenschaftsträgern wird durch die Formel  $\frac{d}{d^2 + 2rd}$  bestimmt, die vereinfacht  $\frac{1}{2-d}$  ergibt. Aus dieser Formel ergibt sich, dass falls die Anlage in einer Bevölkerung selten ist ( $d$  nähert sich 0), so werden in der Bevölkerung nahezu doppelt so viele weibliche dominante Eigenschaftsträger wie männliche vorhanden sein. Kommt die Anlage häufiger vor, wird das Überwiegen für das weibliche Geschlecht vermindert. Stellen die männlichen Eigenschaftsträger die Hälfte der männlichen Bevölkerung dar, so werden die weiblichen Eigenschaftsträger  $\frac{1}{2}$  der männlichen ausmachen, d. h.  $\frac{3}{4}$  des weiblichen Teiles der Bevölkerung darstellen. Ist schliesslich die Anlage äusserst häufig in der Bevölkerung vorkommend ( $d$  nähert sich 1), so werden die männlichen und weiblichen Eigenschaftsträger so gut wie die gesamte Bevölkerung ausmachen und ungefähr gleich häufig vorkommen. Das Verhalten wird durch das obenstehende Diagramm veranschaulicht (Abb. 8).

Eine Voraussetzung, die wir oben die ganze Zeit über angewandt haben, ist, dass Männer und Frauen für sich die Hälfte der Bevölkerung ausmachen. In Wirklichkeit gilt diese Voraussetzung nur approximativ. Die Proportion zwischen beiden Geschlechtern ist ja insoferne etwas abweichend, als unter den Neugeborenen die Knaben ein schwaches Übergewicht haben. Später verschiebt sich die Proportion so, dass in den höheren Altersklassen die relative Anzahl der Knaben mehr und mehr abnimmt, bis schliesslich das Verhältnis umgekehrt ist und wir in höheren Altersgruppen ein Überwiegen der Frauen erhalten. Die Abweichung von der angenommenen Proportion zwischen beiden Geschlechtern ist aber indessen so klein, dass sie für die Probleme, um die es sich hier handelt, kaum eine Rolle spielen dürfte. Wünscht man in einem gegebenen Fall sorgfältigere Berechnungen auszuführen, so stösst es ja andererseits auf keine Schwierigkeit, die obenstehenden Formeln in Hinsicht auf die von

der hier angenommenen Proportion zwischen beiden Geschlechtern abweichende Proportion zu modifizieren.

## Die Zusammensetzung der Population bei monohybrider Triallelie.

Bei monohybrider Triallelie kommen Gene vor, welche die Eigenschaften, um die es sich hier handelt, in Paaren bedingen und bei der Bildung dieser Genpaare sind in der Population drei verschiedene Arten von Genen vorhanden. Nach einer Berechnung im Vorhergehenden (S. 25), können wir bei dieser Sachlage sechs verschiedene Arten von Individuen erhalten, nämlich drei verschiedene Arten von Homozygoten und drei verschiedene Arten von Heterozygoten. Wir nehmen an, dass es sich um ein  $R$ -Gen mit der Wahrscheinlichkeit  $r$  in der Bevölkerung, ein  $D$ -Gen mit der Wahrscheinlichkeit  $d$  in der Bevölkerung und ein  $I$ -Gen mit der Wahrscheinlichkeit  $i$  in der Bevölkerung handelt. Es ist also  $r + d + i = 1$ . Wir erhalten dann folgende verschiedene Arten von Individuen in folgenden Frequenzen:

$RR$ -Individuen mit der Wahrscheinlichkeit $\dots r^2$ ,	welche die Eigenschaft 0 und die Frequenz $p$ in der Bevölkerung haben.
$DR$ -Individuen mit der Wahrscheinlichkeit $\dots 2rd$	} welche die Eigenschaft A und die Frequenz $q$ in der Bevölkerung haben.
$DD$ -Individuen mit der Wahrscheinlichkeit $\dots d^2$	
$RI$ -Individuen mit der Wahrscheinlichkeit $\dots 2ri$	} welche die Eigenschaft B und die Frequenz $s$ in der Bevölkerung haben.
$II$ -Individuen mit der Wahrscheinlichkeit $\dots i^2$	
$DI$ -Individuen mit der Wahrscheinlichkeit $\dots 2di$ ,	welche die Eigenschaft AB und die Frequenz $1-p-q-s$ haben.

Wir haben oben angenommen, dass es sich um zwei dominante und eine rezessive Anlage sowie um Individuen handelt, welche die beiden dominanten Anlagen haben, und von beiden Arten von Individuen, die nur eine dominante Anlage besitzen, eine abweichende Beschaffenheit aufweisen. Dies stellt selbstverständlich keineswegs die einzige Möglichkeit dar. Die Möglichkeit ist auch vorhanden, dass eine Anlage rezessiv ist, eine andere Anlage dominant über die rezessive und eine dritte Anlage, sowohl über die rezessive wie auch über die zweite dominante Anlage dominant ist. Wir behandeln



nun indessen die erste Möglichkeit und nehmen ausserdem an, dass die verschiedenen Eigenschaftsträger, die Frequenz in der Population haben, die wir oben im Schema angenommen haben. Aus diesem Schema werden dann folgende Gleichungen erhalten:

$$r^2 = p$$

$$2rd + d^2 = q$$

$$2ri + i^2 = s$$

$$2di = 1 - p - q - s$$

Aus diesen Gleichungen wird erhalten:

$$r = \sqrt{p} \quad (4)$$

und durch Einsetzen dieses Wertes in die zweite und dritte Gleichung:

$$d = \sqrt{p+q} - \sqrt{p} \quad (5)$$

$$i = \sqrt{p+s} - \sqrt{p} \quad (6)$$

Ausserdem wissen wir, dass

$$r + d + i = 1. \quad (7)$$

Da wir nun die Werte für  $d$  und  $i$  kennen, können wir die Frequenz der Gruppe  $AB$  berechnen, die ja gemäss unserem Schema  $2di$  ist. Dies ergibt die Gleichung:  $s = 1 + p + q + 2\sqrt{p} - 2\sqrt{p+q} - 2\sqrt{p(p+q)}$ . Durch einen Vergleich dieser Summe mit der empirisch gefundenen Frequenz dieser Gruppe, kann man also kontrollieren, ob eine Bevölkerung, die aus vier verschiedenen Arten von Eigenschaftsträgern in angegebenen Frequenzen besteht, möglicherweise durch monohybride Triallelie entstanden ist. Eine andere Kontrolle erhalten wir aus der Gleichung  $r + d + i = 1$ . Ist eine Vererbung nach diesem Mechanismus vorhanden, sollen also die aus den Gleichungen berechneten Werte der Genfrequenzen insgesamt 1 ergeben.

Bei monohybrider Triallelie haben wir auch die Möglichkeit, die wir vorher erwähnt haben, zu beachten, nämlich dass eine Anlage

$R$  mit der Frequenz  $r$  den beiden übrigen gegenüber rezessiv ist, eine Anlage  $D_2$  mit der Frequenz  $d_2$  sowohl über die erste wie auch über die zweite Anlage, die  $D_1$  genannt wird und eine Frequenz  $d_1$  besitzt, dominiert. Bei dieser Sachlage erhalten wir also drei verschiedene Arten von Eigenschaftsträgern in der Bevölkerung. Wir erhalten dann folgende Arten von Individuen mit folgenden Frequenzen:

$RR$ -Individuen mit der Wahrscheinlichkeit $\dots r^2$ ,	welche die Eigenschaft 0 und die Frequenz $p$ in der Bevölkerung haben.
$D_1R$ -Individuen mit der Wahrscheinlichkeit $\dots 2rd_1$	} welche die Eigenschaft A und die Frequenz $q$ in der Bevölkerung haben.
$D_1D_1$ -Individuen mit der Wahrscheinlichkeit $\dots d_1^2$	
$D_2R$ -Individuen mit der Wahrscheinlichkeit $\dots 2rd_2$	} welche die Eigenschaft B und die Frequenz $1-p-q$ in der Bevölkerung haben.
$D_2D_2$ -Individuen mit der Wahrscheinlichkeit $\dots d_2^2$	
$D_1D_2$ -Individuen mit der Wahrscheinlichkeit $\dots 2d_1d_2$	

Mit Hilfe dieser Formeln werden folgende Gleichungen erhalten:

$$r^2 = p$$

$$2rd_1 + d_1^2 = q$$

$$2rd_2 + d_2^2 + 2d_1d_2 = 1 - p - q$$

Wir wissen, dass

$$r + d_1 + d_2 = 1 \quad (8)$$

Aus diesen Gleichungen wird erhalten:

$$r = \sqrt{p} \quad (9)$$

und durch Einsetzen dieses Wertes in die zweite Gleichung

$$d_1 = \sqrt{p+q} - \sqrt{p} \quad (10)$$

sowie durch Einsetzen in die dritte Gleichung

$$d_2 = 1 - \sqrt{p+q} \quad (11)$$

und dies ist die einzige Kontrolle, welche in diesem Falle ausgeführt werden kann.

Haben wir also drei verschiedene Arten von Eigenschaftsträgern in einer Bevölkerung, liegt es am nächsten zu prüfen, ob die Frequenz der Typen mit der Annahme einer monohybriden Diallelie mit intermediärer Erbllichkeit übereinstimmt. Aus der Frequenz der Individuen, für die am meisten Anlass besteht, sie als Homozygoten zu betrachten, wird die Frequenz der betreffenden Anlagen berechnet und die Summe dieser Frequenzen soll 1 sein. Aus diesen Frequenzen kann man ferner die Frequenz der Heterozygoten berechnen. In diesen beiden Punkten (Übereinstimmung zwischen berechneter und gefundener Heterozygotenfrequenz sowie, dass die Summe der Frequenz der Anlagen  $= 1$  sein soll) kann man nun kontrollieren, ob die Zusammensetzung der Population innerhalb der Grenzen des mittleren Fehlers mit dieser Annahme übereinstimmt. Findet man nun, dass dies nicht der Fall ist, kann die Vermutung entstehen, dass eine monohybride Triallelie mit doppelter Dominanz vorliegt. Um zu kontrollieren, ob dieser Mechanismus vorliegt, berechnet man die Frequenz der drei Anlagen. Die Summe dieser Frequenzen soll dann 1 werden. Zeigt sich, dass dies nicht der Fall ist, spricht dies dagegen, dass eine monohybride Triallelie mit doppelter Dominanz vorliegt.

Findet man hingegen eine Übereinstimmung, so bedeutet dies indessen nicht, dass eine monohybride Triallelie mit doppelter Dominanz bzw. eine monohybride Diallelie mit intermediärer Erbllichkeit vorliegt. Es kann sich um einen verwickelteren Vererbungsgang handeln, der aber zufallsbedingt die geforderte Übereinstimmung mit dem einfacheren Mechanismus ergibt. Ein endgültiger Beweis kann erst durch die Analyse der Verwandten der Eigenschaftsträger erhalten werden.

### Die Zusammensetzung der Population bei dihybrider Diallelie.

Bei dihybrider Diallelie werden die betreffenden Eigenschaften durch zwei Anlagepaare bedingt. Wir nehmen an, dass im ersten Anlagepaar das Gen  $R_1$  die Wahrscheinlichkeit  $r_1$  und das Gen  $D_1$  die Wahrscheinlichkeit  $d_1$  hat. Im zweiten Anlagepaar nehmen wir an, dass das Gen  $R_2$  die Wahrscheinlichkeit  $r_2$  und das Gen  $D_2$  die Wahrscheinlichkeit  $d_2$  aufweist. Die Gene können sich nun zu folgenden verschiedenen Arten von Gentypen kombinieren:

$R_1 R_1 R_2 R_2$ mit der Frequenz	$r_1^2 r_2^2$	welche die Eigenschaft A und die Frequenz $p$ in der Bevölkerung aufweisen.
$R_1 R_1 R_2 D_2$ mit der Frequenz	$2 r_1^2 r_2 d_2$	} welche die Eigenschaft A und die Frequenz $q$ in der Bevölkerung haben.
$R_1 R_1 D_2 D_2$ mit der Frequenz	$r_1^2 d_2^2$	
$R_1 D_1 R_2 R_2$ mit der Frequenz	$2 r_1 d_1 r_2^2$	} welche die Eigenschaft B und die Frequenz $s$ in der Bevölkerung haben.
$D_1 D_1 R_2 R_2$ mit der Frequenz	$d_1^2 r_2^2$	
$R_1 D_1 R_2 D_2$ mit der Frequenz	$4 r_1 d_1 r_2 d_2$	} welche die Eigenschaft AB und die Frequenz $1 - p - q - s$ in der Bevölkerung haben.
$R_1 D_1 D_2 D_2$ mit der Frequenz	$2 r_1 d_1 d_2^2$	
$D_1 D_1 R_2 D_2$ mit der Frequenz	$2 d_1^2 r_2 d_2$	
$D_1 D_1 D_2 D_2$ mit der Frequenz	$d_1^2 d_2^2$	

Wir haben oben angenommen, dass es sich um zwei Anlagepaare handelt, von welchen jedes aus einer rezessiven und einer dominanten Anlage zusammengesetzt ist, sowie angenommen, dass die beiden dominanten Anlagen bei gleichzeitiger Anwesenheit keineswegs übereinander dominieren, sondern eine Eigenschaft oder einen Eigenschaftskomplex ergeben, der von den Eigenschaften abweicht, welche durch eine einzige dominante Anlage in Hetero- oder Homozygotenform bedingt ist. Kennen wir, gemäss des oben stehenden Schemas, die Frequenz der verschiedenen Eigenschaftsträger in der Bevölkerung, so erhalten wir folgende Gleichungen, zu deren Ableitung die Beziehungen  $r_1 + d_1 = 1$ ,  $r_2 + d_2 = 1$  also  $d_1 = 1 - r_1$ ,  $d_2 = 1 - r_2$  benutzt werden.

$$r_1^2 \cdot r_2^2 = p$$

$$r_1^2 (1 - r_2^2) = q$$

$$r_2^2 (1 - r_1^2) = s$$

$$(1 - r_1^2) (1 - r_2^2) = 1 - p - q - s$$

aus den ersten drei dieser Gleichungen ergibt sich, dass

$$p = (p + q) (p + s)$$

ist. Die Auflösung nach  $r_1$ ,  $r_2$  u. s. w. ergibt

$$r_1 = \sqrt{\frac{p}{s + p}} = \sqrt{p + q} \quad (12)$$

und

$$r_2 = \sqrt{\frac{p}{q + p}} = \sqrt{p + s} \quad (13)$$

sowie

$$d_1 = 1 - \sqrt{\frac{p}{s+p}} \quad (14)$$

und

$$d_2 = 1 - \sqrt{\frac{p}{q+p}} \quad (15)$$

Ausserdem wissen wir, dass

$$p(1-p-q-s) = r_1^2(1-r_2^2)r_2^2(1-r_1^2) = qs \quad (16)$$

sowie

$$\begin{aligned} (q+1-p-q-s)(s+1-p-q-s) &= [r_1^2(1-r_2^2) + \\ &+ (1-r_1^2)(1-r_2^2)][r_2^2(1-r_1^2) + (1-r_1^2)(1-r_2^2)] = \\ &= (1-r_2^2)[r_1^2 + (1-r_1^2)](1-r_1^2)[r_2^2 + (1-r_2^2)] = \\ &= (1-r_2^2)(1-r_1^2) = 1-p-q-s \end{aligned} \quad (17)$$

Die letzten Bedingungsgleichungen für die Verhältnisse erlauben eine Kontrolle der Werte von  $r_1$  bzw.  $d_1$  und  $r_2$  bzw.  $d_2$ , die aus den vorhergehenden Gleichungen berechnet wurden.

Wir haben weiter oben über die Zusammensetzung der Bevölkerung bei dihybrider Diallelie mit zwei dominanten Anlagen, von denen keine über die andere dominiert, berichtet. Wir haben aber auch mit der Möglichkeit einer doppelten Dominanz zu rechnen, d. h. dass zwei rezessive und zwei dominante Anlagen vorhanden sind und die eine dominante Anlage über die zweite dominiert. Wir erhalten dann nach der folgenden Tabelle nur drei verschiedene Arten von Eigenschaftsträgern, wobei wir annehmen, dass die Anlage  $d_1$  über alle übrigen Anlagen dominiert.

$R_1 R_1 R_2 R_2$ mit der Frequenz	$r_1^2 r_2^2$	welche die Eigenschaft 0 und die Frequenz $p$ in der Bevölkerung haben.
$R_1 R_1 R_2 D_2$ mit der Frequenz	$2 r_1^2 r_2 d_2$	} welche die Eigenschaft A und die Frequenz $q$ in der Bevölkerung haben.
$R_1 R_1 D_2 D_2$ mit der Frequenz	$r_1^2 d_2^2$	
$R_1 D_1 R_2 R_2$ mit der Frequenz	$2 r_1 d_1 r_2^2$	} welche die Eigenschaft B und die Frequenz $1-p-q$ in der Bevölkerung haben.
$D_1 D_1 R_2 R_2$ mit der Frequenz	$d_1^2 r_2^2$	
$R_1 D_1 R_2 D_2$ mit der Frequenz	$4 r_1 d_1 r_2 d_2$	
$R_1 D_1 D_2 D_2$ mit der Frequenz	$2 r_1 d_1 d_2^2$	
$D_1 D_1 R_2 D_2$ mit der Frequenz	$2 d_1^2 r_2 d_2$	
$D_1 D_1 D_2 D_2$ mit der Frequenz	$d_1^2 d_2^2$	

Kennen wir gemäss des oben stehenden Schemas die Frequenz der verschiedenen Eigenschaftsträger in der Bevölkerung, erhalten wir folgende Gleichungen. Wir benutzen dann, dass  $r_1 + d_1 = 1$  und folglich  $d_1 = 1 - r_1$  sowie, dass  $r_2 + d_2 = 1$  und folglich  $d_2 = 1 - r_2$ .

$$r_1^2 \cdot r_2^2 = p$$

$$r_1^2 (1 - r_2^2) = q$$

$$r_2^2 (1 - r_1^2) + (1 - r_1^2) (1 - r_2^2) = 1 - r_1^2 = 1 - p - q$$

was ergibt

$$r_1 = \sqrt{p+q} \quad (18)$$

und

$$r_2 = \sqrt{\frac{p}{p+q}} \quad (19)$$

sowie

$$d_1 = 1 - \sqrt{p+q} \quad (20)$$

und

$$d_2 = 1 - \sqrt{\frac{p}{p+q}} \quad (21)$$

## Die Zusammensetzung der Population bei polymerer Diallelie.

Bei dieser Form von Vererbung handelt es sich um eine grössere oder geringere Anzahl alleler Genpaare, in welchen jedes Gen auf gleichartige Weise wie die anderen Gene wirkt, weswegen also dieses Gen durch ein (oder mehrere) anderes Gen ersetzt werden kann, ohne dass dies irgend einen feststellbaren Unterschied im Hinblick auf die Eigenschaftsbeschaffenheit des Individuums bedingt.

Wir nehmen an, dass es sich um die Genpaare  $D_1 R_1; D_2 R_2; D_3 R_3 \dots D_m R_m$  handelt, welche die Frequenz  $d_1 r_1; d_2 r_2; d_3 r_3 \dots d_m r_m$  haben. Kombinieren sich die Gene zufallsbedingt zu Gameten, so erhalten wir die Genkombinationen, die sich aus der folgenden Ausdruck ergeben:

$$(d_1 + r_1) \cdot (d_2 + r_2) \cdot (d_3 + r_3) \dots (d_m + r_m).$$

Nimmt man dann an, dass die Gameten sich zufallsbedingt miteinander vereinen, so erhalten wir die Frequenzen der Zygotenkombinationen durch Multiplikation des oben stehenden Ausdruckes mit sich selbst. Die verschiedenen Zygotenkombinationen werden also die Frequenzen bekommen, die aus der folgenden Ausdruck erhalten werden:

$$[(d_1 + r_1) \cdot (d_2 + r_2) \cdot (d_3 + r_3) \dots (d_m + r_m)]^2$$

Nehmen wir nun an, dass die verschiedenen Gene eine genau ebenso grosse Wirkung haben, so wird eine Zygote mit nur *R*-Genen die Grundbeschaffenheit haben, während hingegen eine Zygote mit einem *D*-Gen und im übrigen *R*-Genen sozusagen um eine Einheit von der Grundbeschaffenheit abweicht; ferner kann man sagen, dass eine Zygote mit zwei *D*-Genen zwei Einheiten von der Grundbeschaffenheit abweicht u. s. w. Wir nehmen nun ausserdem an, dass alle *D*-Gene gleich häufig vorkommen (Frequenz = *d*) und alle *R*-Gene ebenfalls dieselbe Frequenz (= *r*) besitzen. In diesem Falle vereinfacht sich der oben stehende Ausdruck auf

$$(r + d)^{2m}$$

Ist  $r = d = \frac{1}{2}$ , d. h. kommen die *R*- und *D*-Gene gleich häufig vor, so erhalten wir eine binomiale Verteilung. Individuen, die nur *R*-Gene besitzen, werden relativ selten vorkommen und Individuen, die nur *D*-Gene besitzen, sind ebenfalls verhältnismässig selten. Zwischen diesen Extremen erhalten wir eine sogenannte binomiale Verteilung deren graphische Darstellung sich bei einer grossen Anzahl von Eigenschaften der Gausschen Fehlerkurve annähert. Eigenschaften, welche durch einen solchen Vererbungsmechanismus bedingt werden, müssen also eine Verteilung ergeben, die mit einer Normalkurve ziemlich nahe übereinstimmt. Wie die Verteilung im übrigen aussieht, beruht in erster Linie darauf, ob es sich um streng erbliche Eigenschaften handelt, welche nicht durch das Milieu beeinflusst werden, oder ob das Milieu eine grössere oder geringere Variabilität verursacht. Im ersten Falle werden wir eine Verteilung mit scharfen Grenzen zwischen den Klassen erhalten. Wir erhalten ein Polygon, das mit irgend einer binomialen Verteilung übereinstimmt und aus der Anzahl der scharf voneinander getrennten Klassen kann man in diesem Falle berechnen, um wie viele polymere

Anlagen es sich handelt. Handelt es sich z. B. um drei Anlagepaare, so erhalten wir in diesem Falle folgende Frequenz der verschiedenen 7 Klassen 1 : 6 : 15 : 20 : 15 : 6 : 1.

Dieser Spezialfall dürfte indessen so selten sein, dass er in Wirklichkeit kaum vorkommt. Erstens hat wohl das Milieu auf die Eigenschaften, um die es sich hier handelt, immer irgend eine, wenn auch geringe Wirkung. Man kann erwarten, dass sich diese Wirkung nach einer Wahrscheinlichkeitsgleichung für jede erblich verschiedene Klasse von Eigenschaftsträgern verteilt. Die Bevölkerung wird dann eine Verteilung nach einer Wahrscheinlichkeitsfunktion ergeben, die aus mehreren Wahrscheinlichkeitsverteilungen zusammengesetzt wird. Eine derartige zusammengesetzte Kurve ist in der Regel nicht aufzulösen, ohne dass man eine ganze Reihe willkürlicher Annahmen macht. Durch eine nähere Analyse des Erblichkeitsganges durch Familien und Geschlechter kann man aber einige Anhaltspunkte für eine solche Aufteilung erhalten.

Wir sind indessen bereits in dem letzteren Falle von stark schematisierten Ausgangspunkten ausgegangen. Es ist kaum wahrscheinlich, dass die *R*- und *D*-Gene gleich häufig vorkommen. Kommen die *D*-Gene häufiger vor und handelt es sich um eine geringere Anzahl polymerer Gene, so haben wir eine mehr oder minder schiefe Verteilung zu erwarten. Handelt es sich hingegen um eine grosse Anzahl von Genen oder haben die Gene in den verschiedenen Paaren Frequenzen, die in entgegengesetzte Richtung gehen (kommt also in einem allelen Paar das *D*-Gen häufiger vor als das *R*-Gen, in einem anderen allelen Paar das *R*-Gen häufiger als das *D*-Gen), so wird die Schiefeit der Verteilung geringer werden und vielleicht verschwinden. Wir sind davon ausgegangen, dass *D*-Gene über *R*-Gene dominieren. Man hat natürlich auch die Möglichkeit mit intermediärer Vererbung zu rechnen, d. h. dass *RD*-Individuen eines bestimmten Anlagepaares eine Zwischenstellung zwischen *RR*- und *DD*-Individuen einnehmen.

Wir können also zusammenfassend sagen, dass wir bei polymerer polyhybrider Diallelie wahrscheinlich eine Verteilung erhalten, die mehr oder minder gut mit einer Wahrscheinlichkeitskurve übereinstimmt, aber eine mehr oder minder ausgeprägte schiefe Verteilung aufweisen kann. Man kann sich selbstverständlich auch vorstellen, dass die Verteilung einen Exzess aufweist. Dieser kann möglicherweise auf Grund der modifizierenden Wirkung des Milieufaktors entstanden sein. Haben die Faktoren eine variierend kräftige Wir-



kung, so kann man sich auch vorstellen, dass sie auf erblichen Faktoren beruht.

Im übrigen soll hervorgehoben werden, dass man dasselbe Ergebnis zu erwarten hat, wenn es sich um eine polymere Polyallelie handelt. Es dürfte sich erübrigen, die Gedankengänge über diese näher zu entwickeln, da diese analog mit denen sind, die anlässlich unserer vorhergehenden Überlegung aufgebaut wurden.

Als Beispiel für Eigenschaften, von welchen man annehmen kann, dass sie einen Vererbungsmechanismus von dieser Art haben, kann die menschliche Körperlänge angeführt werden. Durch anthropologische Untersuchungen weiss man, dass die Körperlänge in Populationen eine Verteilung aufweist, die ziemlich gut mit einer Normalkurve übereinstimmt. In dem Material der meisten Autoren findet man auch eine unbedeutend schiefe Verteilung und mitunter auch einen weniger bedeutenden Exzess. Es ist ohne weiteres klar, dass die Variabilität teilweise durch Milieufaktoren bedingt wird. Es wurde aber nachgewiesen, dass der grösste Teil der Variabilität von Vererbungsfaktoren her stammt. Da man trotzdem eine solche Verteilung erhält, muss dies darauf beruhen, dass die Vererbungsfaktoren auch eine Verteilung nach einer Normalkurve verursachen, was mit ziemlich grosser Sicherheit darauf hinweist, dass es sich um einen polymeren Vererbungsmechanismus handelt. Gegenwärtig ist es aber unmöglich, das Problem näher zu analysieren und zu entscheiden, um wie viele Vererbungsfaktoren es sich handelt, eine wie grosse Wirkung jeder derselben besitzt, sowie mit welcher Frequenz die entsprechenden Gene in einer bestimmten Bevölkerung vorkommen.

## Die Zusammensetzung der Population bei komplizierteren Formen der Vererbung.

Im Vorhergehenden haben wir die Zusammensetzung der Population bei monohybrider Diallelie, geschlechtsgebundener monohybrider Diallelie, monohybrider Triallelie, dihybrider Diallelie und polymerer Diallelie behandelt. Nach dem Muster der Berechnungsarten, die wir hierbei angewandt haben, dürfte es auf keine grösseren Schwierigkeiten stossen, Gleichungen für etwas kompliziertere Vererbungsformen aufzustellen. Generelle Formeln für die verschiedenen möglichen Vererbungsformen, die vorstellbar sind, aufzustellen,

dürfte aus praktischen Gesichtspunkten kaum lohnend sein. Man muss sich daran erinnern, dass wir mit einer unendlichen Anzahl von verschiedenen Möglichkeiten zu rechnen haben. Es kann sich erstens um eine willkürliche Anzahl alleler Gene (Polyhybridität) handeln. Zweitens kann man sich für jedes Paar eine willkürliche Anzahl von Genen vorstellen, von denen nur zwei in einem bestimmten Paar enthalten sind (Polyallelie). Drittens haben wir im Hinblick auf den Charakter der Eigenschaft mit der Möglichkeit zu rechnen, dass eine einfache oder in verschiedenem Grade vielfache Dominanz vorliegt, ferner mit der Möglichkeit, dass eine inter- oder extramediäre Vererbung vorhanden ist. Schliesslich hat man auch an die Möglichkeit eine geschlechtsgebundene Vererbung zu denken.

Kennt man die Anzahl der Eigenschaftsträger in einer Bevölkerung im Hinblick auf erblich zusammengehörende Eigenschaften, so kann man mit Hilfe der Formeln, die wir oben gefunden haben oder mit Hilfe für den besonderen Fall aufgestellten Formeln wenigstens manchmal zu dem einfachsten vorstellbaren Vererbungsmechanismus gelangen, der mit der Frequenz der Eigenschaftsträger übereinstimmt. Dass in der Wirklichkeit kein verwickelterer Mechanismus vorliegt, ist aber deswegen nicht bewiesen. Bestimmten Werten kann eben so gut durch eine einfachere als auch durch eine komplizierte Vererbungsformel für die Bevölkerung Genüge getan werden. Unter solchen Verhältnissen ist es selbstverständlich am richtigsten durch Untersuchungen von Familienkreisen und Geschlechtern zu versuchen, eine Vorstellung zu erhalten, welcher Vererbungsmechanismus in einem gegebenen Fall vorliegt und zu versuchen, das Ergebnis durch einen Vergleich mit den Verhältnissen in der Population in ihrer Gesamtheit zu verifizieren, falls hierzu Material zur Verfügung steht. Nun ist aber die Sachlage die, dass wir für die meisten Eigenschaften keine populationsstatistischen Angaben besitzen. Aus diesem Grunde ist ein solcher Vergleich nur ausnahmsweise möglich und dies stellt einen weiteren Grund dafür dar, dass wir es nicht der Mühe wert erachten, auf die verschiedenen Möglichkeiten der Zusammensetzung der Populationen aus Erblichkeit Gesichtspunkten näher einzugehen. Indessen haben wir aber bei der verhältnismässig kurz gefassten und unvollständigen Untersuchung, über die wir weiter oben berichtet haben, gewisse allgemeine Schlüsse erhalten, die teils an und für sich damit rechnen können, Interesse zu finden und die teils auch bei der Aufstellung

von Formeln, die in der Methodik der Vererbungsforschung angewandt werden, benutzt werden können. Um diese Schlüsse besser bewerten zu können, ist es indessen notwendig, näher zu ermitteln, in welchem Ausmasse die grundlegende Voraussetzung, dass in einer Bevölkerung eine Panmixie stattfindet, als mit zuverlässiger Genauigkeit nachgewiesen anzusehen ist.

## Die Einwirkung der Mutationen auf die Zusammensetzung der Population bei Panmixie.

Wir wissen aus der zoologischen und botanischen Vererbungs-forschung, dass Mutationen mehr oder minder selten aus unbekanntem Anlass bei verschiedenen Organismen auftreten. Über das Vorkommen von Mutationen beim Menschen wissen wir indessen kaum etwas oder nichts. Wir haben keine exakte Vorstellung über ihre Frequenz, wir wissen aber, dass sie sehr seltene Phänomene sein müssen; anderenfalls würde ja unser Beobachtungsmaterial nicht so spärlich sein. HALDANE (1935) hat versucht die Mutationsfrequenz für gewisse Eigenschaften zu berechnen und erhielt den Wert  $10^{-5}$ . Bereits aus der Tatsache, dass Mutationen sehr selten vorkommen müssen, folgt, dass ihre Bedeutung für die Zusammensetzung der Bevölkerung, insoferne man dieses Problem nur für eine kürzere Zeitspanne betrachtet, sehr gering sein dürfte. Vergleichen wir die Zusammensetzung der Bevölkerung mit dem Zwischenraum von einer oder einigen Generationen, so kann man ohne weiteres davon ausgehen, dass eventuell auftretende Mutationen so selten sind, dass sie keinen deutlichen Unterschied zwischen den beiden Generationen verursachen können. Hieraus folgt aber keineswegs, dass Mutationen eine gleichgültige Erscheinung darstellen, wenn man die Entwicklung einer Bevölkerung während eines sehr langen Zeitraums untersuchen will. Trotzdem Mutationen selten sind, ist es aber nicht ausgeschlossen, dass sie gegebenenfalls ziemlich deutliche Veränderungen verursachen.

Wir gehen von einer Bevölkerung mit Panmixie aus und nehmen an, dass Mutationen in erster Linie in Form von Heterozygoten auftreten, welche die mutative Anlage  $R$  besitzen. Wir nehmen ferner an, dass die Frequenz der Mutationen der Gene während einer Generation  $\mu$  ist. Ausserdem nehmen wir an, dass die mutierten Individuen dieselbe Aussicht haben sich zu vermehren wie die anderen sowie, dass unter den mutierten Individuen keinerlei Mutationen

stattfinden. Rechnet man die Ausgangsgeneration als erste Generation, so werden die mutierten Gene in der  $n$ -ten Generation eine Frequenz unter den Genen haben, die in der unten stehenden Formel angegeben wird

$$1 - (1 - \mu)^n. \quad (22)$$

Die Frequenz der nicht mutierten Gene wird

$$(1 - \mu)^n. \quad (23)$$

Geht man davon aus, dass  $\mu$  einen sehr niedrigen Wert besitzt, so wird der Ausdruck näherungsweise für die mutierten Gene

$$1 - e^{-\mu n} \quad (24)$$

und für die nicht mutierten Gene

$$e^{-\mu n} \quad (25)$$

wobei  $n$  die Anzahl Generationen und  $\mu$  die Mutationsfrequenz per Generation bedeutet, wie im Vorhergehenden.

Aus der Formel ergibt sich, dass falls die Mutationsfrequenz sich Null nähert, so wird die Zusammensetzung der Gene der Bevölkerung anfänglich nicht merkbar verändert. Erst nach einer sehr langen Zeit wird die Wirkung sichtbar und nach einer unendlichen Anzahl von Generationen sind alle Gene mutiert. Nun hat man in der Wirklichkeit nicht mit unendlichen Zeiträumen zu rechnen, ferner besitzen die mutierten Individuen, bei welchen sich die Anlage manifestiert, oft nicht dieselbe Aussicht sich fortzupflanzen wie die, bei welchen die Eigenschaft fehlt, welche durch das mutierte Gen bedingt wird. In einigen Fällen haben die Eigenschaftsträger eine grössere Aussicht sich fortzupflanzen und die Veränderung geht dann schneller vor sich, als es die oben stehende Formel angibt. In anderen Fällen haben die neuen Eigenschaftsträger eine geringere Aussicht sich fortzupflanzen und der Prozess geht dann langsamer vor sich oder bleibt in einer Gleichgewichtslage stehen oder die Mutation stirbt aus. Probleme dieser Art haben, wie oben angedeutet, ein sehr grosses Interesse für die Erklärung der Entstehung neuer Arten. Für das menschliche Geschlecht besitzen diese Probleme aber eine geringere Bedeutung. Unsere Möglichkeiten, die Zukunft vorauszu-  
sehen, sind sehr begrenzt, weswegen es kaum Interesse besitzt, einen

Versuch zu unternehmen, um zu berechnen, was nach einer grösseren Anzahl von Generationen geschehen sein wird. Man kann verschiedener Auffassung sein, was man unter einer grösseren Anzahl von Generationen versteht. Deswegen soll hervorgehoben werden, dass falls man als sog. „historische“ Zeit 6 000 Jahre nimmt und rechnet, dass jede Generation 25 Jahre umfasst, so entspricht diese Zeit 240 Generationen. Bei einer Fliege, mit einem zeitlichen Abstand zwischen den Generationen von einem Monat, würde dies ungefähr 20 Jahren entsprechen. Im weiteren Verlauf dieser Arbeit soll die Frage nach der Bedeutung der Mutationen in der Besprechung der Einwirkung der Selektion beim Menschen berührt werden.

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# Die Wirkung der Selektion auf eine Bevölkerung.

## Totale Selektion.

Viele Ursachen können es bedingen, dass Individuen mit einer bestimmten Zusammensetzung der Erbmasse eine niedrigere Fruchtbarkeit haben als die Bevölkerung im Durchschnitt. Die Zusammensetzung der Erbmasse kann dazu führen, dass sie vor dem geschlechtsreifen Alter sterben, dass die Individuen steril werden oder dass ihr Wert vom Gesichtspunkt der Ehe aus besonders niedrig wird. Sterilisierungsmassnahmen oder andere in rassenhygienischer Hinsicht vorgenommene Massnahmen können Individuen mit einem bestimmten Vererbungstypus an der Fortpflanzung verhindern u. s. w.

Um die prinzipielle Wirkung der Selektion zu veranschaulichen, gehen wir davon aus, dass alle Eigenschaftsträger von einem bestimmten Typus vollständig daran verhindert werden, sich fortzupflanzen (totale Selektion) und ferner davon, dass eine monohybride Diallelie vorliegt.

Es ist dann ohne weiteres klar, dass falls die *dominanten* Eigenschaftsträger an der Fortpflanzung verhindert werden, so wird die Eigenschaft sofort in der Bevölkerung ausgerottet. Dies ist ohne Zweifel die Ursache dafür, dass erblich bedingte, schwere Krankheiten, die sich regelmässig im Alter vor der Geschlechtsreife äussern und dominant vererbt werden, praktisch genommen, nicht vorkommen. Solche Eigenschaftsträger kommen ja selten oder niemals dazu sich fortzupflanzen und können deswegen ihre Anlage auf keine Nachkommen übertragen. Ein praktisches Beispiel für diese Verhältnisse stellt die juvenile amaurotische Idiotie dar. Kinder, die an dieser erblichen Krankheit leiden, beginnen im Alter von 6—8 Jahren Veränderungen in Form von Abstumpfung des Intellekts und einer allmählich eintretenden Erblindung zu zeigen. Der Prozess schreitet dann fort, so dass die Kinder sterben, bevor sie

erwachsen sind. Würde diese Eigenschaft als eine monohybride, dominante Eigenschaft vererbt, würde sie nach einer Generation in der Bevölkerung verschwunden sein. Sie kann nicht auf diese Weise vererbt werden, weil Eigenschaftsträger vorkommen, wenn auch in niedriger Frequenz.

Für die rezessiven Eigenschaftsträger ist die Sachlage eine völlig andere. Wir nehmen an, dass es sich um ein  $R$ -Gen handelt, das die Frequenz  $r$  hat. Die rezessiven Eigenschaftsträger, die  $RR$ -Individuen, haben folglich die Frequenz  $r^2$ . Letztere werden nun daran gehindert, sich fortzupflanzen. Die Gameten, welche die nächste Generation entstehen lassen würden, werden nun um diese Anzahl vermindert und ihre Frequenz wird also  $1 - r^2$ ; unter diesen Gameten finden sich  $r - r^2$   $R$ -Gameten. In der folgenden Generation ergibt sich also die Frequenz der  $R$ -Gameten  $r_2$ :

$$r_2 = \frac{r - r^2}{1 - r^2} = \frac{r}{1 + r}$$

Hieraus folgt, dass in der nächsten Generation  $RR$ -Individuen mit der Frequenz

$$r_1 = \left( \frac{r}{1 + r} \right)^2 \text{ auftreten.}$$

Diese Eigenschaftsträger werden nun wieder verhindert sich fortzupflanzen und die nächste Generation wird also von der folgenden Anzahl von Gameten produziert:

$$1 - \left( \frac{r}{1 + r} \right)^2$$

Unter diesen Gameten befindet sich die folgende Anzahl von  $R$ -Gameten:

$$\frac{r}{1 + r} - \left( \frac{r}{1 + r} \right)^2$$

Setzen wir die Rechnung auf diese Weise fort, so finden wir, dass die Frequenz der  $R$ -Gameten in der dritten Generation,  $r_3$ , die folgende ist:

$$r_3 = \frac{r}{1 + 2r}$$



sowie, dass schliesslich in der  $n$ . Generation ihre Frequenz  $r_n$  ist

$$r_n = \frac{r}{1 + (n-1)r}$$

Es kann leicht gezeigt werden, dass wenn die Formel für  $n$  Generationen gilt, sie auch für  $n+1$  Generationen Gültigkeit besitzt.

Wenden wir nun diese Formel für die Berechnung der Zusammensetzung der Zygoten in einer Population nach  $n$  Generationen an, so erhalten wir folgende Formeln:

Frequenz der rezessiven Eigenschaftsträger  $RR$ :

$$r_n^2 = \frac{r^2}{[1 + (n-1) \cdot r]^2} \quad (26)$$

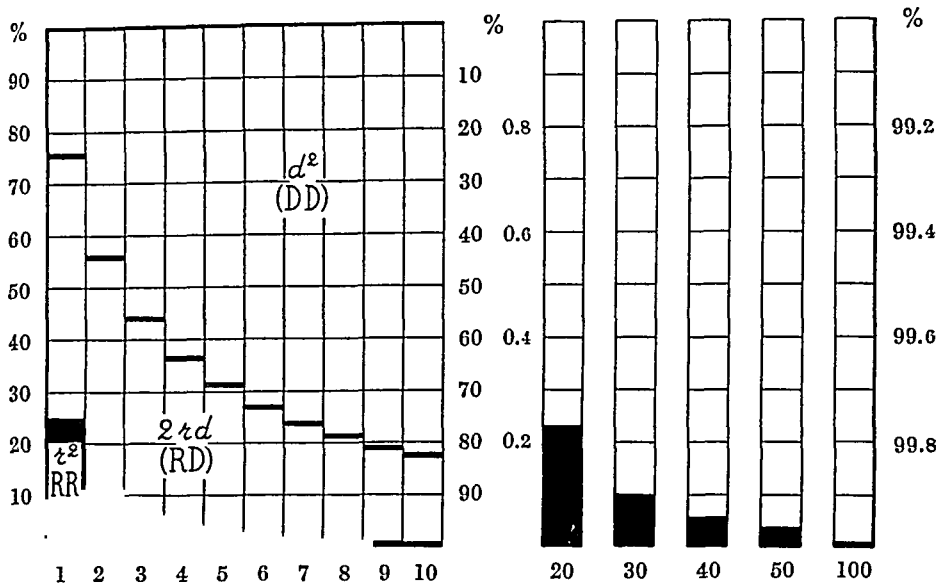
Frequenz der Heterozygoten  $RD$ :

$$2r_nd_n = \frac{2r[1 + (n-2) \cdot r]}{[1 + (n-1) \cdot r]^2} \quad (27)$$

Frequenz der dominanten Homozygoten  $DD$ :

$$d_n^2 = \frac{[1 + (n-2) \cdot r]^2}{[1 + (n-1) \cdot r]^2} \quad (28)$$

Mit Hilfe dieser Formeln wurde das Diagramm in Abb. 9 konstruiert und die Tabelle 2 aufgestellt. Das Diagramm zeigt, wie die Prozentwerte für die verschiedenen Arten von Individuen in einer Population verschoben werden, wenn die rezessiven Eigenschaftsträger immer daran verhindert werden, sich fortzupflanzen. In der Ausgangsgeneration besitzen die rezessiven Eigenschaftsträger die Frequenz 25 %. Sie werden anfänglich sehr schnell ausgerottet. Bereits in der vierten Generation ist ihre Frequenz auf 4 % gesunken. Gehen wir hingegen von einer Bevölkerung mit 0.1 % Eigenschaftsträgern aus (30. Generation im Diagramm), so sinkt ihre Frequenz in 10 Generationen nicht voll auf die Hälfte (0.06 %). Es ist auch auffällig, dass die Wirkung auf die Heterozygoten bedeutend schwächer ist. Zwischen der vierten und neunten Generation wird die Anzahl der rezessiven Homozygoten von 4 % auf 1 % vermindert, während hingegen die Heterozygoten nur von 32 % auf 18 %. Nach 100 Generationen haben wir nur 0.01 % Eigenschaftsträger, aber 1.96 % oder beinahe 200 mal



Generation

Abb. 9. Abnehmende Häufigkeit der rezessiven Merkmalsträger bei verhin-  
deter Fortpflanzung derselben.

TABELLE 2.

Häufigkeit von Individuen verschiedener Genotypen in aufeinanderfolgenden  
Generationen bei monohybrider Diallelie und totaler Selektion rezessiver Eigen-  
schaftsträger. Vgl. Formel 26, 27 und 28.

Generation	RR	RD	DD
1	0.2500	0.5000	0.2500
2	0.1111	0.4444	0.4444
3	0.0625	0.3750	0.5625
4	0.0400	0.3200	0.6400
5	0.0278	0.2778	0.6944
6	0.0204	0.2449	0.7347
7	0.0156	0.2188	0.7656
8	0.0123	0.1975	0.7901
9	0.0100	0.1800	0.8100
10	0.0083	0.1653	0.8264
20	0.0023	0.0907	0.9070
30	0.0010	0.0624	0.9365
40	0.0006	0.0476	0.9518
50	0.0004	0.0384	0.9612
100	0.0001	0.0196	0.9803

so viele Heterozygoten. Dies bedeutet also, dass die Selektion anfänglich auf die Eigenschaftsträger eine kräftige Wirkung ausübt, dass diese aber niemals völlig ausgerottet werden, sowie, dass die Wirkung auf die Heterozygoten bedeutend schwächer ist. Auch nach einer sehr grossen Anzahl von Generationen und einer Selektion von der stärksten vorstellbaren Art können sie in einigen Prozent in der Bevölkerung noch vorhanden sein. Dies stimmt mit dem Schluss überein, zu dem wir vorher gelangt sind, dass nämlich, falls die rezessiven Eigenschaftsträger sehr selten sind, die Heterozygoten doch relativ zahlreich vorkommen.

Handelt es sich um eine Selektion, bei der die Eigenschaftsträger vollständig an der Fortpflanzung verhindert werden, so denkt man gerne an Sterilisierungsmassnahmen. Die Sachlage hierfür ist ja die, dass man eine kleine Gruppe von Mitbürgern als weniger geeignete Eltern einer kommenden Generation ansieht, weswegen man sie verhindert sich fortzupflanzen. Handelt es sich bei dieser Sachlage darum, die zu erwartende Wirkung zu berechnen, so muss man selbstverständlich erst ermitteln, wie die Fortpflanzung der Gruppe wahrscheinlich ausgefallen wäre, wenn die Sterilisierungsmassnahmen nicht vorgenommen worden wären. Ferner muss man den Vererbungstypus für die Eigenschaften, um die es sich handelt, kennen. Schliesslich muss man selbstverständlich auch wissen, welche Frequenz die verschiedenen Eigenschaftsträger besitzen, welche die Gruppe zusammensetzen. Die Gruppe ist ja sicher nicht einheitlich (z. B. besteht die erblich bedingte Blindheit aus mindestens etwa 10 Typen). Besteht die Gruppe z. B. aus einer sehr grossen Anzahl verschiedener Eigenschaftsträger, von denen jede Art für sich sehr selten ist, so ist es klar, dass man nicht Berechnungen unter der Voraussetzung ausführen darf, dass die Gruppe homogen ist. Die Wirkung der Selektion, gerichtet gegen eine äusserst seltene Eigenschaft, ist ja sehr nahe Null. Richtet man die Massnahmen gegen mehrere verschiedene Arten seltener Eigenschaftsträger, so wird der gesamte Effekt ebenfalls sehr klein und keineswegs so gross, wie er erwartet werden kann, falls die Gruppe homogen gewesen wäre und aus einer einzigen Art von Eigenschaftsträgern bestanden hätte.

## Partielle Selektion.

Ist nun die Selektion weniger effektiv, so dass nur ein Teil der Eigenschaftsträger an der Fortpflanzung verhindert wird (partielle Selektion), so gelten im Prinzip dieselben Schlüsse, wie sie oben gezogen wurden. Der Prozess geht nur langsamer vor sich.

Wir untersuchen in erster Linie die *monohybride Rezessivität*. Wird in jeder Generation ein Anteil  $= k$  der Eigenschaftsträger an der Fortpflanzung verhindert und bedingt dies, dass in der  $n$ -ten Generation der Gehalt an einer rezessiven Anlage, die ursprünglich die Frequenz  $r$  aufwies auf  $r_n$  vermindert wird, so wird in dieser Generation die Frequenz der Anlage um  $kr_n^2$  vermindert und eine entsprechende Verminderung tritt auch für die gesamte Bevölkerung ein. Deswegen werden die Eigenschaftsträger in der nächsten Generation  $R_{n+1}$  die Frequenz

$$r_{n+1}^2 = \left[ \frac{r_n - kr_n^2}{1 - kr_n^2} \right]^2 \text{ erhalten.} \quad (29)$$

Mit Hilfe dieser Rekursionsformel können die Werte für aufeinander folgende Ausdrücke berechnet werden. Die Formel drückt aus, dass die Frequenz der rezessiven Anlage anfänglich schneller abnimmt und dann langsamer (vgl. Tab. 3 und Abb. 27 und 29). Theoretisch gesehen muss der Prozess mit sich bringen, dass nach einer unendlich langen Zeit die Anlage aus der Bevölkerung verschwindet. Man kann dies auf folgende Weise veranschaulichen. Nehmen wir an, dass in einer Urne schwarze und weisse Kugeln vorhanden sind. Wir nehmen nun paarweise Kugeln aus der Urne und legen sie in eine neue Urne. In dem Fall, in welchem man nun zwei schwarze Kugeln in einem Paar erhält, wird das Paar weggeworfen, und zwar in einer gewissen Frequenz. Man erhält dann einen niedrigeren Gehalt an schwarzen Kugeln in der Urne Nr. 2 als in der Nr. 1. Führt man solche Überführungen in neue Urnen nach und nach aus, müssen schliesslich alle schwarzen Kugeln paarweise so oft zusammengetroffen sein, dass sie alle weggeworfen wurden. Nach einer unendlich langen Zeit müssen deswegen die schwarzen Kugeln verschwunden sein (mit einer Ausnahme; die letzte schwarze Kugel kann, wenn die Anzahl ungerade ist, niemals in einem Paar schwarzer Kugeln enthalten sein und deswegen nicht weggeworfen werden).

TABELLE 3.

Effekt von partieller Selektion in aufeinanderfolgenden Generationen bei rezessiver, monohybrider Diallelie, wenn die Selektion rezessiver Eigenschaftsträger von variierender Stärke ist. Vgl. Formel 29.

Generation	$k = 0.01$	$k = 0.05$	$k = 0.10$	$k = 0.25$	$k = 0.50$	$k = 0.75$
0	0.010000	0.010000	0.010000	0.010000	0.010000	0.010000
1	0.009982	0.009910	0.009821	0.009554	0.009116	0.008686
2	0.009964	0.009821	0.009646	0.009136	0.008342	0.007613
3	0.009946	0.009733	0.009475	0.008745	0.007661	0.006726
4	0.009928	0.009647	0.009309	0.008377	0.007059	0.005984
5	0.009910	0.009562	0.009147	0.008032	0.006524	0.005353
6	0.009892	0.009478	0.008989	0.007707	0.006047	0.004825
7	0.009874	0.009395	0.008835	0.007401	0.005620	0.004367
8	0.009856	0.009313	0.008685	0.007112	0.005236	0.003971
9	0.009838	0.009232	0.008539	0.006840	0.004890	0.003626
10	0.009821	0.009152	0.008396	0.006583	0.004576	0.003323
15	0.009735	0.008767	0.007732	0.005487	0.003379	0.002253
20	0.009650	0.008405	0.007142	0.004639	0.002594	0.001633

Mathematisch hat J. B. S. HALDANE (1924) auf folgende Art bewiesen, dass der Prozess zu einem Verschwinden der Anlage führen muss. Der Unterschied zweier aufeinander folgender Termen ist

$$r_{n+1} - r_n = -k r_n^2 \frac{1 - r_n}{1 - k r_n^2}$$

Die Zeit, welche eine Generation umfasst, ist, falls der Prozess unendlich lange vorsichgehen kann, unendlich klein im Verhältnis zu der Zeit, welche verflossen ist. Es handelt sich darum, eine Gleichung zu finden, welche den obenstehenden Ausdruck als Differential hat;

$$\therefore \frac{dr_n}{dn} = -k r_n^2 \frac{(1 - r_n)}{1 - k r_n^2}$$

$$\int_0^n dn = -\frac{1}{k} \int_{r_0}^{r_n} \frac{1 - k r_n^2}{r_n^2 (1 - r_n)} dr_n$$

Bei Lösung des Integrals erhält man:

$$n = \frac{1}{k} \left[ \frac{1}{r_n} - \frac{1}{r_o} + \log \frac{r_o}{r_n} + (1-k) \log \frac{1-r_n}{1-r_o} \right] \quad (30)$$

Aus diesem Ausdruck geht hervor, dass wenn  $r_n \rightarrow 0$ ,  $n$  sich dem  $\infty$  nähert.

Man kann ohne HALDANES Integral ganz elementar zeigen, dass

$$r_{n+1} < \frac{r_1}{1 + n r_1}$$

ist. Darüber hinaus gilt noch

$$r_{n+1} > \frac{n}{n+1} r_n.$$

Also konvergieren die  $r_n$  sehr langsam gegen Null.

Es bleibt noch übrig, den Verlauf des Prozesses bei *monohybrider Dominanz* zu untersuchen. Werden in jeder Generation  $\frac{k}{1+k}$  der Eigenschaftsträger verhindert, sich fortzupflanzen und pflanzen sich also  $\frac{1}{1+k}$  fort, so wird die rezessive Anlage, falls sie nach  $n$  Generation die Frequenz  $r_n$  besitzt, in der folgenden Generation die folgende Frequenz haben:

$$r_{n+1} = \frac{r_n^2 + \frac{1}{1+k} r_n d_n}{r_n^2 + \frac{1}{1+k} d_n^2 + \frac{1}{1+k} 2 r_n d_n},$$

$$\therefore r_{n+1} = \frac{r_n + k r_n^2}{1 + k r_n^2},$$

$$\text{und } r_{n+1}^2 = \left[ \frac{r_n + k r_n^2}{1 + k r_n^2} \right]^2, \quad (31)$$

was derselbe Ausdruck ist, den wir weiter oben für die partielle Selektion bei Rezessivität mit Zeichenveränderungen gefunden haben.

Die Frequenz der dominanten Eigenschaftsträger wird

$$d_{n+1}^2 + 2 r_{n+1} d_{n+1} = 1 - \left[ \frac{r_n + k r_n^2}{1 + k r_n^2} \right]^2 \quad (32)$$

TABELLE 4.

Effekt von partieller Selektion in aufeinanderfolgenden Generationen bei rezessiver, monohybrider Diallelie und bei verschiedener Stärke der Selektion dominanter Eigenschaftsträger. Vgl. Formel 32.

Generation	$\frac{k}{1+k} = 0.1$	$\frac{k}{1+k} = 0.25$	$\frac{k}{1+k} = 0.5$	$\frac{k}{1+k} = 0.75$
0	0.0100	0.0100	0.01000	0.0100000
1	0.0090	0.0075	0.00501	0.00252361
2	0.0081	0.0056	0.00252	0.00063190
3	0.0073	0.0043	0.00126	0.00015819
4	0.0066	0.0032	0.00064	0.00004000
5	0.0059	0.0024	0.00032	0.00001000
6	0.0053	0.0018	0.00016	0.00000250
7	0.0048	0.0014	0.00008	0.00000063
8	0.0043	0.0010	0.00004	0.00000016
9	0.0039	0.0008	0.00002	0.00000004
10	0.0035	0.0006	0.00001	0.00000001

Die Formel zeigt, dass partielle Selektion weit stärkeren Effekt bei dominanten als bei rezessiven Eigenschaftsträgern hat. Vgl. Tab. 4.

Wir untersuchen schliesslich die Wirkung der Selektion bei einer verwickelteren Vererbungsart. Vererbt sich eine Anlage als eine dihybride dominante Eigenschaft und nimmt man an, dass die eine Anlage die Frequenz  $d'$  und die zweite  $d''$  besitzt, ferner dass  $d' = id''$ , so wird bei totaler Selektion die erste Anlage nach  $n$  Generationen die Frequenz

$$d'_n = \frac{d'}{1 + (n-1)\frac{d'}{i}} \quad (33a)$$

haben und die zweite Anlage eine Frequenz von

$$d''_n = \frac{d''}{1 + (n-1)id''} \quad (33b)$$

Ist  $i = 1$ , d. h.  $d' = d'' = d$ , so wird der Ausdruck für beide Anlagen

$$d_n = \frac{d}{1 + (n-1)d} \quad (34)$$

was bedeutet, dass der Prozess auf dieselbe Art wie bei monohybrider Rezessivität verläuft. Aus der Formel ergibt sich ferner

dass falls die eine Anlage häufiger vorkommt als die andere, so wird die Anlage mit der häufigeren Frequenz in ihrer Frequenz langsam abnehmen und die seltene Anlage verhältnismässig schnell. In dem extremen Fall, nämlich dass die eine Anlage in der gesamten Bevölkerung vorkommt und die andere Anlage eine niedrigere Frequenz aufweist, wird die letztere nach einer Generation verschwinden, worauf die häufiger vorkommende Anlage die Frequenz unverändert beibehalten wird, die sie nachher hat. Bei anderen komplizierteren Vererbungsformen werden die Verschiebungen langsamer vorsichgehen als bei Rezessivität. Bei dihybrider Rezessivität muss z. B., wenn beide Anlagen gleich häufig vorkommen, der Prozess wesentlich langsamer vorsichgehen als bei monohybrider Rezessivität. Kommt die eine Anlage häufig und in der gesamten Bevölkerung vor, und ist die andere Anlage weniger frequent, so werden die Verschiebungen wie bei monohybrider Rezessivität verlaufen.



## Selektion und Mutationen.

Treten nun in einer Bevölkerung Mutationen auf und sind die Eigenschaftsträger, die hierdurch entstehen, einer Selektion ausgesetzt, so muss der Prozess sich einer Gleichgewichtslage nähern, wenn die Anzahl der Mutationen der Anzahl von Eigenschaftsträgern entspricht, die auf Grund von Selektion verschwunden sind. Z. B. muss bei monohybrider Rezessivität, wenn die Anlage die Frequenz  $r$  und die Mutationen die Frequenz  $\mu$  haben, eine Gleichgewichtslage eintreten, wenn

$$r^2 = 2\mu$$

Die Frequenz der Heterozygoten,  $2rd$ , wird dann

$$2rd = 2\sqrt{2\mu}(1 - \sqrt{2\mu})$$

Werden nun  $k$  von den rezessiven Eigenschaftsträgern an der Fortpflanzung verhindert, so erhalten wir folgenden Ausdruck:

$$r^2 = \frac{2\mu}{k} \tag{35}$$

$$2rd = 2\sqrt{\frac{2\mu}{k}} \cdot \left(1 - \sqrt{\frac{2\mu}{k}}\right) \tag{36}$$

Der Ausdruck in der Klammer ist selbstverständlich so nahe 1, dass er vernachlässigt werden kann.

Wird die Frequenz von Eigenschaftsträgern aus der Gleichgewichtslage verschoben, so wird eine immer langsamere Rückkehr zur Gleichgewichtslage in den folgenden Generationen vor sich gehen. Dies wird erst nach unendlicher Zeit erreicht. Das Problem wird später behandelt werden, wenn wir Prozesse diskutieren, welche ein einmal erreichtes Gleichgewicht stören können.

Wir haben bisher vorausgesetzt, dass Selektion und Mutationen in einer Bevölkerung stattfinden, in welcher im übrigen eine Pan-

mixie vorhanden ist. Kommen andere Abweichungen von der Panmixie vor, kann dies auf die Prozesse einwirken und wir werden im folgenden aus diesem Grunde wieder die Selektion und Mutationen in verschiedenen Zusammenhängen berühren.

Der erste, der versucht hat die Selektion zu berechnen, ist, so weit ich ermitteln konnte, WARREN (1917), der einfache numerische Berechnungen ausgeführt hat. Die grundlegende Arbeit auf diesem Gebiete wurde von J. B. S. HALDANE (1924—1932) ausgeführt. HALDANE behandelt das Problem vom Standpunkt der Evolution aus und mit einem verwickelten mathematischen Apparat. Die Arbeit wurde in einer wenig gelesenen Zeitschrift veröffentlicht und wurde zum Teil deswegen anfänglich nicht in einem weiteren Kreis beachtet. Ohne Kenntnis der Arbeit von HALDANE hat Verf. früher einige grundlegende Formeln angegeben und ihre Anwendung auf den Menschen besprochen (DAHLBERG 1926, ferner DAHLBERG und HULTKRANTZ 1927). Später haben andere Autoren, ohne Kenntnis der Arbeit von HALDANE, ebenfalls versucht das Problem mathematisch zu analysieren, so z. B. BODEWIG 1932—33.

## Die Wirkung der Verwandtschaftsehe auf eine Bevölkerung.

Durch eine einfache Überlegung kann man sich klar machen, dass die Verwandtschaftsehe die Entstehung von Homozygotie begünstigt und der Heterozygotie entgegenwirkt. Entsteht eine Anlage durch eine Mutation bei einem bestimmten Menschen in rezessiver Form, so kann die Anlage in homozygoter Form nur durch eine Ehe zwischen den Nachkommen von Mutanten zusammentreffen, also durch eine Verwandtschaftsehe. Die Homozygoten, die in späteren Generationen vorhanden sein werden, werden also alle auf Verwandtschaftsehen beruhen, wenn auch die Verwandtschaft zwischen den Individuen nicht immer so stark ist, dass sie nachweisbar ist.

Man muss sich indessen daran erinnern, dass nachdem eine Population aus Familien und Geschlechtern zusammengesetzt ist, in der Voraussetzung, dass eine Panmixie vorliegt, enthalten ist, dass auch eine gewisse Anzahl von Verwandtschaftsehen stattfinden werden. Ist die Bevölkerung im Verhältnis zu den einzelnen Geschlechtern gross, so enthält die Forderung für eine Panmixie eine verhältnismässig kleine Anzahl von näheren Verwandtschaftsehen. Ist hingegen die Population im Verhältnis zu den Geschlechtern klein, so bedeutet die Forderung für Panmixie, dass eine grössere Anzahl von Verwandtschaftsehen vorkommen soll. Es muss daran erinnert werden, dass die Annahme, dass eine Entwicklung stattfindet, bedeutet, dass vielleicht alle Individuen verwandt sind, notabene, wenn man annimmt, dass sich das menschliche Geschlecht aus einer einzigen Mutation oder aus einer geringeren Anzahl von Mutationen entwickelt hat, zwischen deren Nachkommen Kreuzungen stattgefunden haben. Man gelangt zum gleichen Schluss, wenn man die Ahnen einer bestimmten Person für eine grössere Anzahl zurückliegender Generationen berechnet. Man gelangt dann schnell zu Werten, die weit die vorstellbare Grösse der Bevölkerung der Erde im betreffenden Zeitpunkt überschreiten. So sind z. B. die Ahnen ei-

ner Person, wenn man annimmt, dass keine Verwandtschaftsehen stattgefunden haben, für 30 vorhergehende Generationen mehr als 1 000 Millionen und für 100 Generationen mehr als  $10^{30}$ . Dies zeigt, dass Ahnenverluste, d. h. Verwandtschaftsehen in sehr grosser Ausdehnung während früherer Generationen stattgefunden haben müssen. Wenn auch Ehen zufallsbedingt geschlossen werden, so müssen wir doch eine grössere oder geringere Anzahl von Verwandtschaftsehen erwarten. Die Frage ist nun: Wie gross ist die Abweichung, welche durch Verwandtschaftsehe verursacht wird, von dem, was man bei Panmixie zu erwarten hat, wenn Verwandtschaftsehen in grösserem oder geringerem Ausmasse vorkommen als man bei Panmixie zu erwarten hat. Um dieses Problem zu lösen, müssen wir uns in erster Linie ein Mass für die Wirkung, welche die Verwandtschaftsehe hat, verschaffen.

### Die Zusammensetzung der Nachkommen in Verwandtschaftsehen.

Wir berechnen in erster Linie die Nachkommenzusammensetzung in einer Geschwisterehe und bei monohybrider Diallelie. Wir nehmen an, dass das rezessive Gen  $R$  die Frequenz  $r$  und also das dominante Gen  $D$  die Frequenz  $1 - r$  hat. Ein bestimmtes Gen unter den vier Genen der Ausgangspersonen hat die Aussicht  $\frac{1}{2}$  ein bestimmtes Kind zu erreichen und wiederum die Aussicht  $\frac{1}{2}$  ein Kindeskind zu erreichen (vgl. Abb. 10). Insgesamt ist also die Aussicht

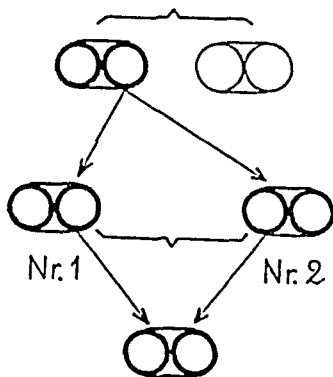


Abb. 10. Ehe zwischen Geschwistern. Die mit zwei Linien vereinigten Kreise stellen ein Genpaar vor.

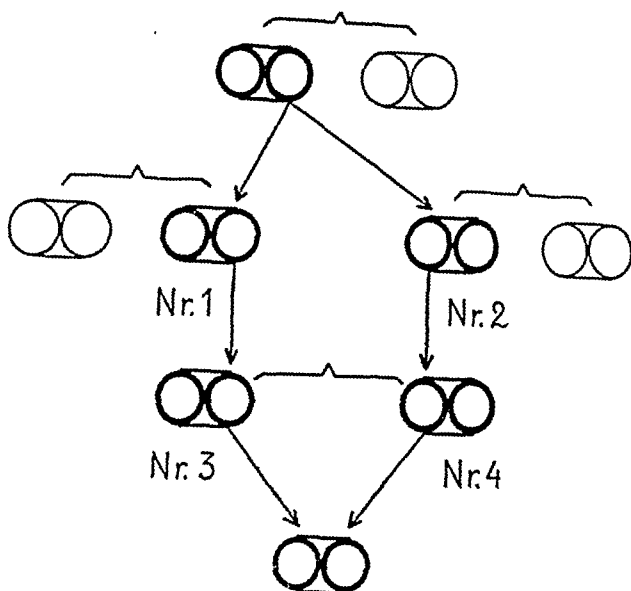


Abb. 11. Ehe zwischen Geschwisterkindern. Die mit zwei Linien vereinigten Kreise stellen ein Genpaar vor.

$\frac{1}{4}$ . Die Aussicht, dass das Gen über ein anderes Kind ebenfalls Kindeskind erreichen wird, ist auch  $\frac{1}{4}$ . Die Aussicht, dass es hier mit sich selbst zusammentreffen wird ist also  $\frac{1}{4} \cdot \frac{1}{4} = \frac{1}{16}$ . Wir haben aber vier Gene, die als Ausgangspunkte für die Entstehung eines solchen Homozygoten dienen können. Die Aussicht wird deshalb vierfach oder  $4 \cdot \frac{1}{16} = \frac{1}{4}$ . Bei einem Viertel der Kinder trifft also eines der Ausgangsgene zu einem Homozygoten zusammen und in  $r$  Fällen ist der Ausgangspunkt ein  $R$ -Gen. Wir erhalten also einen rezessiven Eigenschaftsträger auf Grund dessen, dass der Ausgangspunkt mit sich selbst in  $\frac{r}{4}$  der Fälle zusammentrifft. In der übrigen  $\frac{3}{4}$  der Fälle trifft irgend eines der Gene zufälligerweise mit einem anderen Gen derselben Art zusammen, so dass ein rezessiver Homozygot entsteht. Dies muss mit derselben Frequenz geschehen, welche ein solches Zusammentreffen in der Bevölkerung hat, d. h. in  $r^2$  von diesen  $\frac{3}{4}$  der Fälle. Insgesamt erhalten wir also Eigenschaftsträger unter den Nachkommen in Geschwisterehen in

$$\frac{1}{4}r + \frac{3}{4}r^2 \text{ Fällen.} \quad (37)$$

Auf analoge Weise wird die wahrscheinliche Frequenz der Eigenschaftsträger unter den Nachkommen einer Vetternehe berechnet (vgl. Abb. 11). Ein bestimmtes Gen hat die Aussicht  $\frac{1}{2}$  eines der

Kinder zu erreichen,  $\frac{1}{4}$  ein Kindeskind und die Aussicht  $\frac{1}{8}$  ein Kindeskindeskind zu erreichen. In einer Vetternehe ist also die Aussicht für das Zusammentreffen eines bestimmten Genes mit sich selbst  $\frac{1}{8} \cdot \frac{1}{8} = \frac{1}{64}$ . Wir haben indessen vier Gene bei den gemeinsamen Eltern, die für ein solches Zusammentreffen als Ausgangspunkt dienen können und diese haben die Wahrscheinlichkeit  $r$   $R$ -Gene zu sein. Die Wahrscheinlichkeit dafür, dass ein Gen mit sich selbst zusammentreffen soll, so dass ein rezessiver Eigenschaftsträger entsteht, ist also  $4 r \cdot \frac{1}{8} \cdot \frac{1}{8} = \frac{1}{16} r$ . Bei den übrigen  $\frac{1}{8}$  der Nachkommen entstehen Eigenschaftsträger durch zufallsbedingtes Zusammentreffen von Genen mit im Durchschnitt derselben Aussicht, wie dies in der Bevölkerung geschieht, d. h. in  $r^2$  Fällen. Insgesamt wird also die Frequenz der rezessiven Eigenschaftsträger unter den Nachkommen in einer Vetternehe

$$\frac{r}{16} + \frac{15 r^2}{16} \quad (38)$$

und die Frequenz dominanter Eigenschaftsträger

$$d^2 + r d - \frac{1}{16} r d. \quad (39)$$

## Die Zusammensetzung der Population bei Verwandtschaftsehe in grossen Populationen.

Wir nehmen an, dass wir es mit einer sehr grossen Bevölkerung zu tun haben. In dieser sind also die einzelnen Familien und Geschlechter im Verhältnis zur Bevölkerung verschwindend klein, weswegen wir bei Panmixie nur eine verschwindend kleine Anzahl von Verwandtschaftsehen zu erwarten haben. Die Anzahl der Verwandtschaftsehen nähert sich also bei dieser Sachlage und Panmixie 0. Wird eine Eigenschaft durch rezessive monohybride Diallelie bedingt, erhalten wir also, wenn das  $R$ -Gen die Frequenz  $r$  hat, rezessive Eigenschaftsträger mit der Frequenz  $r^2$ .

Wir nehmen nun an, dass in einer Bevölkerung nur Vetternehen geschlossen werden. In diesem Falle erhält die Bevölkerung die Zusammensetzung  $\frac{1}{16} r + \frac{15}{16} r^2$ , gemäss der Formel, die wir oben abgeleitet haben. Die Erhöhung, welche diese unerhört starke Ver-

wandtschaftsehe für die Frequenz der rezessiven Homozygoten bedingt, wird also

$$\frac{1}{16}r + \frac{15}{16}r^2 - r^2$$

was vereinfacht ergibt

$$\frac{1}{16}(r - r^2) = \frac{2rd}{32} \quad (40)$$

Aus diesem Ausdruck ergibt sich, dass die Heterozygoten mit  $\frac{1}{16}$  abnehmen und dass der Hälfte dieser Abnahme  $\frac{1}{32} = 3.13\%$  der Heterozygoten eine ebenso grosse Zunahme der rezessiven bzw. dominanten Homozygoten entspricht. Die Zunahme erreicht das Maximum, wenn  $r = \frac{1}{2}$ . In diesem Falle sind bei Panmixie 25% rezessive Eigenschaftsträger vorhanden. Bei Vetternehe allein werden weitere  $\frac{1}{8} = 1.56\%$  der Bevölkerung Eigenschaftsträger sein und wir erhalten also statt dessen 26.56% Eigenschaftsträger. Ist die Anlage seltener, so wird die Verschiebung, welche diese extreme Verwandtschaftsehe in der Beschaffenheit der Bevölkerung verursacht, noch geringer. Die Verhältnisse werden durch Tabelle 5 und Abb. 12 beleuchtet. Das Diagramm zeigt, wie die Wirkung der Verwandtschaftsehe langsam bis zu einem Maximum bei  $r = \frac{1}{2}$  wächst und dann langsam sinkt. In Abb. 13 wird die Steigerung bei steigendem Prozentwert der Eigenschaftsträger in der Bevölkerung veranschaulicht.

Aus den Diagrammen und der Tabelle ergibt sich, dass auch in dieser extremen Situation, in der wir bei Panmixie keinerlei Verwandtschaftsehen zu erwarten hatten, in der die Bevölkerung aber statt dessen nur Vetternehen einging, nur eine verhältnismässig unbedeutende Verschiebung in der Zusammensetzung der Bevölkerung festzustellen ist. Nun kann ja in der menschlichen Bevölkerung die Verwandtschaftsehe niemals so hohe Grade erreichen. Nach offiziellen statistischen Angaben kann man in grossen Populationen eher mit einer Frequenz von Verwandtschaftsehen von höchstens etwa 1% Vetternehen und 0.075% Ehen zwischen Geschwistern der Eltern und Kindern der Geschwister, rechnen. Auch wenn diese letzteren Ehen nun eine etwas grössere Erhöhung (zweimal so starke) als Vetternehen verursachen, muss man doch damit rechnen, dass Verwandtschaftsehen, mit den Frequenzen und Gra-

TABELLE 5.

Erhöhung des Prozentsatzes rezessiver, monohybrider Merkmalsträger bei steigender Häufigkeit einer rezessiven Anlage in einer Population, wo nur Ehen zwischen Geschwisterkindern geschlossen werden.

Häufigkeit einer Anlage in einer Population	Häufigkeit der Merkmalsträger einer Population bei Panmixie	Erhöhung des Gehaltes an Merkmalsträgern einer Population, wo nur Geschwisterkinderehen stattfinden
0.001	0.0001	0.000619
0.1	0.01	0.00562
0.2	0.04	0.01000
0.3	0.09	0.01313
0.4	0.16	0.01500
0.5	0.25	0.01562
0.6	0.36	0.01500
0.7	0.49	0.01313
0.8	0.64	0.01000
0.9	0.81	0.00562

 $\frac{rd}{16}$ 

0.02

0.01

 $r$  0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Abb. 12. Erhöhung der Häufigkeit monohybrider, rezessiver Merkmalsträger in einer Population, in welcher nur Geschwisterkinderehen geschlossen werden, im Vergleich mit Panmixie bei zunehmendem Anlagengehalt. Der Masstab in vertikaler Richtung ist zehnmal grösser als in horizontaler Richtung, um die Kurve deutlicher zu machen.

 $\frac{rd}{16}$ 

0.02

0.01

 $r^2$  0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Abb. 13. Erhöhung der Häufigkeit monohybrider, rezessiver Merkmalsträger in einer Population, in welcher nur Geschwisterkinderehen geschlossen werden, im Vergleich mit Panmixie bei zunehmendem Prozentsatz von Merkmalsträgern. Der Masstab in vertikaler Richtung ist zehnmal grösser als in horizontaler Richtung, um die Kurve deutlicher zu machen.



den von Verwandtschaftsehen mit denen man angebrachterweise in menschlichen Bevölkerungen rechnen kann, keine nennenswerte Verschiebung der Frequenz einer Eigenschaft in der Bevölkerung verursachen müssen, verglichen mit Panmixie. Die Veränderungen, die man erwarten kann, sind mit anderen Worten von so niedrigem Grad, dass sie kaum auf empirisch—statistischem Wege nachgewiesen werden können. Die Fehlerquellen, die mit jeder Bevölkerungsstatistik unvermeidlich verbunden sind, dürften so gut wie immer eine allzu starke Rolle spielen, um mit Sicherheit solche Differenzen nachweisen zu können, um die es sich hier handelt.

### Die Zusammensetzung der Population bei Verwandtschaftsehe in kleinen Populationen.

Man kann sich vorstellen, dass eine Bevölkerung so klein ist, dass man bei Panmixie eine verhältnismässig grosse Anzahl von Verwandtschaftsehen zu erwarten hat, dass aber solche aus irgend einem Anlass ausbleiben. Wir wählen wieder ein extremes Beispiel. Wir nehmen an, dass es sich um eine Bevölkerung handelt, in welcher man bei Panmixie zu erwarten hat, dass Ehen in einer bestimmten Frequenz  $a$  Geschwisterehen sein sollen. Ist  $a = \frac{1}{10}$  und haben die Familien eine gleich grosse Anzahl heiratsfähiger Kinder und diese Anzahl ist 2, so wird die Bevölkerung aus ungefähr 5 Familien bestehen. Ist die Anzahl der Kinder 3, so besteht die Bevölkerung aus ungefähr 10 Familien und wenn die Kinderanzahl 4 ist, besteht die Bevölkerung aus 15 Familien. In diesen Populationen kommen nun keine Geschwisterehen vor und dies bedingt eine Abweichung von der erwarteten Zusammensetzung der Bevölkerung bei Panmixie. Hat die rezessive Anlage  $R$  die Frequenz  $r$ , so erhält man bei Panmixie  $r^2$  rezessive Eigenschaftsträger. In der Geschwisterehe ist, wie wir vorher gezeigt haben, die Zusammensetzung der Nachkommen  $\frac{1}{4}r + \frac{3}{4}r^2$ . Bei Panmixie besitzen also  $a$  der Bevölkerung diese Zusammensetzung. Wir nehmen an, dass die übrigen  $1 - a$  der Bevölkerung die Zusammensetzung  $x$  haben. Wir erhalten also folgende Gleichung:

$$r^2 = a \left( \frac{1}{4}r + \frac{3}{4}r^2 \right) + (1 - a)x$$

Löst man diese Gleichung auf, so erhält man:

$$x = \frac{4r^2 - ar - 3ar^2}{4(1-a)}$$

Kommen keine Geschwisterchen vor, so erhält die Bevölkerung die Zusammensetzung, welche der Teil der Population hat, in welchem keine Geschwisterchen stattfinden. Wollen wir mit vollständiger Panmixie vergleichen, so erhalten wir also eine Verminderung der Anzahl der rezessiven Eigenschaftsträger  $\Delta x$  aus der Gleichung

$$\Delta x = x - r^2$$

$$\Delta x = \frac{a(r^2 - r)}{4(1-a)} = -\frac{ard}{4(1-a)} \quad (41)$$

Dieser Ausdruck ist 0, wenn  $r = 0$  oder wenn  $r = 1$  ist. Der Ausdruck ist zwischen diesen beiden Werten negativ, was bedeutet, dass die ausgebliebenen Geschwisterchen eine Abnahme der rezessiven Eigenschaftsträger verursachen. Diese Verminderung ist maximal, wenn  $r = \frac{1}{2}$ ; ist  $a$  z. B.  $\frac{1}{10}$ , so beträgt die Verminderung in diesem Falle 1.39%. Wir erhalten also in diesem Falle statt 25% rezessive Eigenschaftsträger 23.61%. Trotzdem es sich also um eine extrem kleine Bevölkerung handelt, ist die maximale Verminderung, welche die ausgebliebenen Geschwisterchen bewirken, kaum bedeutend. Es soll ferner daran erinnert werden, dass falls es sich um seltenere Anlagen handelt, die Erhöhung noch weniger feststellbar ist. Zum Vergleich soll angeführt werden, dass falls man bei Panmixie auf Grund der Grösse der Bevölkerung im Verhältnis zur Familiengrösse eine Geschwisterche in 1% erwartet, so wird deren Ausbleiben eine maximale Verminderung der Anzahl der Eigenschaftsträger auf 0.12% bedingen. Ist die Anzahl der Kinder 2, so wird die Bevölkerung ungefähr 50 Familien oder etwa 100 Individuen umfassen.

Aus diesen Berechnungen ergibt sich, dass ein Ausbleiben von Geschwisterchen einen verhältnismässig unbedeutenden Unterschied an Eigenschaftsträgern, verglichen mit Panmixie, bedingen wird, auch wenn es sich um verhältnismässig kleine Bevölkerungen und ziemlich gewöhnliche Eigenschaften handelt. Die Beispiele, die wir gewählt haben, um dies zu demonstrieren, dürften weit unter dem liegen, was man in menschlichen Populationen zu finden erwartet, abgesehen selbstverständlich von äusserst seltenen Aus-

nahmeverhältnissen. Da die Verwandtschaftsehe sogar für solche Populationen ein gleichgültiges Geschehen ist, dürfte man ohne weiteres behaupten können, dass für die Populationsgrösse, mit der man unter normalen Verhältnissen zu rechnen hat, die Verwandtschaftsehe keine beachtenswerte Rolle spielt. Wie später gezeigt werden soll, hat man eher Anlass anzunehmen, dass die Populationsgrösse (Isolatgrösse) in Westeuropa mehrere hundert Individuen beträgt.

Wir haben unsere Berechnungen nur für Geschwisterehen ausgeführt, und zwar deshalb, weil Geschwisterehen in menschlichen Populationen immer ausbleiben. Ausserdem ist die Wirkung eventueller anderer ausgebliebener Verwandtschaftsehen bedeutend schwächer, als sie für Geschwisterehen ist. Schliesslich kann man sicher erwarten, dass diese anderen Verwandtschaftsehen nicht in grösserem Ausmasse ausbleiben. Es besteht kaum Anlass anzunehmen, dass sich Vettern und Basen in nennenswert grösserem oder geringerem Ausmasse miteinander verheiraten würden als nicht verwandte Personen in einer Bevölkerung (vgl. weiter unten). Wir können deswegen annehmen, dass Vetternehen und andere Verwandtschaftsehen (abgesehen von Geschwisterehen und Ehen zwischen Eltern und Kindern; letztere dürften auf Grund des Altersunterschiedes bei Panmixie sehr selten sein) in vielen Populationen in ungefähr dem Umfange vorkommen müssen, wie man es im Hinblick auf die Grösse der Population bei Panmixie zu erwarten hat. Die Abweichungen (die später diskutiert werden), haben sicher eine sehr geringe Grössenordnung.

### Verwandtschaftsehe und komplizierte Vererbungsmechanismen.

Die Schlüsse, welche gezogen wurden, gelten für monohybride Diallelie, sie haben aber auch für andere rezessive Vererbungsarten Gültigkeit. Man kann zeigen, dass die Wirkung der Verwandtschaftsehe bei dihybrider Diallelie auf die Frequenz gewisser Eigenschaftsträger geringer ist, als wenn eine monohybride Diallelie vorliegt. Die Wirkung ist am geringsten, wenn die beiden Gene gleich häufig vorkommen. Die Wirkung wird stärker, wenn das eine Gen häufiger und das andere seltener vorkommt und wird maximal, wenn das

eine Gen so gut wie bei allen Individuen in der Bevölkerung vorkommt; in letzterem Falle haben wir praktisch genommen eine monohybride Diallelie. Bei höheren Formen von Polyhybridität wird die Wirkung noch schwächer. Man kann die Sache so ausdrücken, dass die Wirkung der Verwandtschaftsehen kräftiger ist, je seltener die Gene sind; bei einer bestimmten Frequenz der Eigenschaftsträger erhält man den höchsten Grad der Seltenheit, wenn eine Monohybridität vorliegt.

Es verbleibt nun die Frage nach der Wirkung der Verwandtschaftsehe bei Dominanz. Bei Monohybridität kann man sagen, dass die Dominanz die Kehrseite der Rezessivität ist. Die Erhöhung an rezessiven Eigenschaftsträgern, die man erhält, kommt auf Kosten der dominanten Eigenschaftsträger zustande. Man muss sich aber auch daran erinnern, dass falls ein rezessives Gen selten ist, das entsprechende dominante Gen häufig vorkommt und umgekehrt. Kommt nun das rezessive Gen häufig vor, so erhalten wir keine statistisch feststellbare Veränderung der Beschaffenheit der Bevölkerung auf Grund von Verwandtschaftsehen. Dies bedeutet, dass wir auch für selten vorkommende dominante Gene ebenfalls keine feststellbare Veränderung der Zusammensetzung der Population durch Verwandtschaftsehe erhalten. Ist ein rezessives Gen selten, so kommt es ebenfalls zu keiner feststellbaren Veränderung der Zusammensetzung der Populationen durch Verwandtschaftsehe, was ebenfalls bedeutet, dass Verwandtschaftsehen für die Zusammensetzung der Populationen gleichgültig sind, auch wenn es sich um gewöhnliche dominante Gene handelt. Dieselben Schlüsse können wir für verschiedene Formen von Polyhybridität ziehen. Die Verwandtschaftsehe ist für die Eigenschaftsträger gleichgültig, welche durch rezessive Gene bedingt werden, und zwar falls diese selten oder häufig vorkommen, und die Verwandtschaftsehe ist deshalb auch für polyhybride dominante Gene gleichgültig. Wir haben bisher Verwandtschaftsehe im Bezug auf einzelne Eigenschaften diskutiert. Indessen beeinflusst die Verwandtschaftsehe gleichzeitig alle seltenen Eigenschaften und die Gesamtwirkung kann nicht ohne weiteres vernachlässigt werden. Wir werden auf diese Fragen in einem späteren Kapitel näher eingehen, in welchem wir die Frequenz der Verwandtschaftsehen unter den Eltern der Eigenschaftsträger behandeln werden.

Im allgemeinen dürfte man die Auffassung haben, dass die Ver-

wandtschaftsehe eine sehr grosse Bedeutung besitzt, und zwar deshalb, weil man in der praktischen Zuchtarbeit eine sehr kräftige Wirkung mit einer intensiven Inzucht erhalten hat. Hierbei hat man durchgehend eine Kreuzung zwischen Geschwistern Generation nach Generation vorgenommen. Bei solchen Kreuzungen verschwinden die Heterozygoten ziemlich schnell, was u. a. von SEWALL WRIGHT (1921) gezeigt wurde. Wenn auch beim Menschen die Verwandtschaftsehe auf prinzipiell dieselbe Weise wirkt, d. h. eine verminderte Heterozygotie bewirkt, ist die Wirkung, die hier gezeigt wurde, ungemein viel geringer, da in der Praxis nur Vetternehen in verhältnismässig geringer Frequenz vorkommen und wiederholte Vetternehen Generation nach Generation zufallsbedingt so selten vorkommen, dass derartigen Kreuzungen jede Bedeutung fehlt.

Formeln für menschliche Populationen wurden vom Verf. angegeben (DAHLBERG 1929). Später hat HOGBEN (1933 a) Formeln für entferntere Grade der Verwandtschaftsehe berechnet. Diese Berechnungen sind schwierig und Hogben hat durch Einführung einer besonderen Rechnungsart (Matrizenrechnung) eine einfache Methode zur Berechnung der Formeln angeführt.

### Verwandtschaftsehen vom Standpunkt der Eigenschaftsträger aus.

Es wurde weiter oben hervorgehoben, dass Verwandtschaftsehen für die Frequenz sehr seltener rezessiver Eigenschaften von entscheidender Bedeutung sind. Aus dieser Feststellung folgt, dass falls man von Trägern seltener Eigenschaften ausgeht, so findet man eine hohe Frequenz Vetternehen unter ihren Eltern. Bereits 1919 hat LENZ eine Formel für die Berechnung der Frequenz, die bei verschiedener Frequenz einer monohybriden diallelen Eigenschaft zu erwarten ist, abgeleitet. Die Ableitung ist aber approximativ, da LENZ teils nur von Heterozygoten ausgeht und die Homozygoten nicht berücksichtigt und teils auch davon absieht, dass man bei Panmixie eine Reihe von Vetternehen zu erwarten hat. Die korrekte Formel für grosse Populationen wurde von DAHLBERG (1929) angegeben.

Wir nehmen an, dass die Anlage die Frequenz  $r$  und die Vetternehen die Frequenz  $c$  haben; ferner dass die Population so gross ist,

dass bei Panmixie keine Vetternehen zu erwarten sind. Die Frequenz der Ehen, die nicht Vetternehen sind, ist dann  $1 - c$ . Nehmen wir an, dass die Population sehr gross ist, so würden praktisch genommen keine Vetternehen vorkommen. In diesem Falle bedeutet  $c$  eine Zunahme der Vetternehen, die hinausgeht über das, was bei Panmixie zu erwarten ist.

Aus Vetternehen stammen Eigenschaftsträger mit folgender Frequenz:

$$\frac{cr}{16} (1 + 15r).$$

In der Population kommen die Eigenschaftsträger mit folgender Frequenz vor:

$$(1 - c)r^2 + \frac{cr}{16} (1 + 15r).$$

Das Verhältnis zwischen den Eigenschaftsträgern, die aus Vetternehen stammen und der gesamten Anzahl von Eigenschaftsträgern  $k$  wird:

$$k = \frac{\frac{cr}{16} (1 + 15r)}{(1 - c)r^2 + \frac{cr}{16} (1 + 15r)};$$

$$\therefore k = \frac{c}{c + \frac{16(1 - c)r}{1 + 15r}} \quad (42)$$

Ist in dieser Formel  $c$  ein sehr kleiner Wert, so kann man  $1 - c = 1$  setzen und nimmt man auch  $1 + 15r = 1$  an (was eine zweifelhafte Approximation darstellt, da, wenn  $15r = 0$ , man auch das Recht besitzen dürfte, auch  $16r = 0$  zu setzen) so erhält die Formel folgendes Aussehen:

$$k = \frac{c}{c + 16r} \quad (43)$$

Dies ist die approximative Formel, welche LENZ abgeleitet hat (vgl. auch HOGBEN 1933 b).

Nimmt man an, dass Vetternehen tatsächlich mit einer bestimmten Frequenz  $c_1$  vorkommen und bei Panmixie mit einer Frequenz

von  $c_2$  zu erwarten sind, so werden die Eigenschaftsträger in folgender Frequenz aus Vetternehen stammen:

$$\frac{c_1 r}{16} (1 + 15 r).$$

In der Population dürfte man bei Panmixie die Eigenschaftsträger mit einer Frequenz von  $r^2$  erwarten. Man erhält aber eine Zunahme der Frequenz der Eigenschaftsträger in der Population mit

$$(c_1 - c_2) \frac{r}{16} (1 + 15 r).$$

Insgesamt ergibt sich also eine Frequenz der Eigenschaftsträger in der Population von:

$$(1 - c_1 + c_2) r^2 + (c_1 - c_2) \frac{r}{16} (1 + 15 r).$$

Deswegen wird das Verhältnis zwischen den Eigenschaftsträgern, die aus Vetternehen stammen und der gesamten Anzahl von Eigenschaftsträgern:

$$k = \frac{\frac{c_1 r}{16} (1 + 15 r)}{(1 - c_1 + c_2) r^2 + (c_1 - c_2) \frac{r}{16} (1 + 15 r)};$$

$$\therefore k = \frac{c_1}{c_1 - c_2 + \frac{16 r (1 - c_1 + c_2)}{1 + 15 r}} \quad (44)$$

Ist  $c_2 = 0$ , d. h. ist die Population so gross, dass die zufallsbedingte Frequenz der Vetternehen, die zu erwarten ist, so klein ist, dass sie vernachlässigt werden kann, so wird die Formel (43) erhalten, wenn man ausserdem, wie früher bereits angenommen,  $1 + 15 r = 1$  setzt, was einer diskutablen Annäherung gleichkommt. Ist  $c_1 = c_2 = c$ , d. h. kommen Vetternehen in der Frequenz, die bei Panmixie zu erwarten ist, vor, so wird die Formel

$$k = \frac{c (1 + 15 r)}{16 r} \quad (45)$$

TABELLE 6.

Prozent Eigenschaftsträger, die von Geschwisterkinderehen herkommen =  $k$ , bei verschiedener Frequenz einer rezessiven, monohybriden, diallelen Eigenschaft RR und bei verschiedener Frequenz von Geschwisterkinderehen in der Bevölkerung =  $c$ . Vgl. Formel 45.

RR	$k$ für			
	$c = 0.1 \%$	$c = 0.25 \%$	$c = 0.5 \%$	$c = 1 \%$
0.000001	6.344	15.860	31.721	63.439
0.00005	0.978	2.444	4.888	9.776
0.0001	0.710	1.797	3.594	7.188
0.0005	0.373	0.933	1.866	3.733
0.001	0.291	0.729	1.457	2.912
0.0025	0.219	0.547	1.094	2.188
0.005	0.182	0.455	0.911	1.821
0.01	0.156	0.391	0.781	1.563
0.05	0.122	0.304	0.609	1.217
0.1	0.114	0.284	0.568	1.135

In Tabelle 6 und Abb. 14 werden Angaben über die Prozentwerte an Eigenschaftsträgern angegeben, die aus Vetternehen bei verschiedener Frequenz einer rezessiven monohybriden diallelen Eigenschaft und bei verschiedener Frequenz von Vetternehen stammen. Die approximative Formel (43) ergibt niedrigere Werte als diejenigen, die man mittels der korrekten Formel (45) erhält, wenn man annimmt, dass Vetternehen in dem Ausmasse vorkommen, das man bei Panmixie erwarten muss. Der Unterschied ist für selten vorkommende Veranlagungen und besonders für kleine Populationen mit hoher Vetternehefrequenz nicht unwesentlich. Wenn z. B. die Anlage bei 1 von 10 000 vorkommt und die Vetternehen 1 % ausmachen, sind gemäss der approximativen Formel 5.88 % der Eltern der Eigenschaftsträger Vettern, während der richtige Wert 7.19 % ist. Die Unterschiede können mit anderen Worten in bestimmten Fällen von Bedeutung sein.

In diesem Zusammenhange soll ferner hervorgehoben werden, dass man eine ähnliche Erhöhung auch bei komplizierteren Vererbungsformen zu erwarten hat. (Bei dihybrider Dominanz ist, wenn die beiden Anlagen gleich häufig sind, die Erhöhung maximal und gleich mit der bei monohybrider Rezessivität; ist die eine Anlage häufig vorkommend, die andere selten, so liegt eine äusserst unbedeutende Abnahme von Vetternehen unter den Eltern vor.)



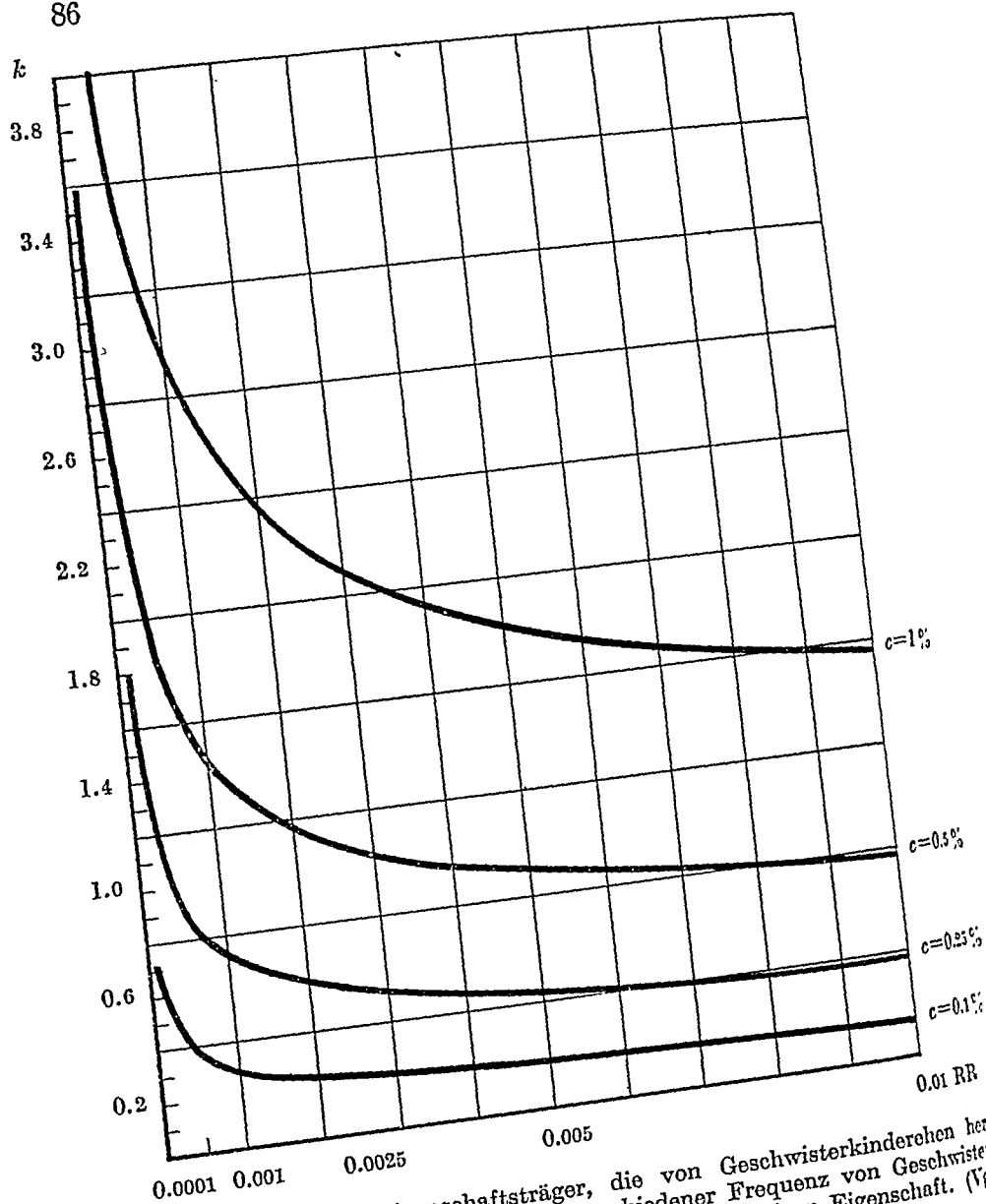


Abb. 14. Prozent Eigenschaftsträger, die von Geschwisterkinderehen herkommen ( $k$ ), in Populationen mit verschiedener Frequenz von Geschwisterkinderehen ( $c$ ) bei verschiedener Frequenz einer rezessiven Eigenschaft. (Vgl. Tabelle 6.)

Will man überhaupt beweisen, dass Erbfaktoren für die Entstehung von Eigenschaften, wie z. B. Krankheiten und ähnliches, die relativ selten sind, eine Rolle spielen, kann man zwei Wege wählen. Man kann eine erhöhte Frequenz der Eigenschaft bei nahen Verwandten zum Eigenschaftsträger (Geschwister, Eltern, Kindern u. s. w.) nachweisen und man kann eine Erhöhung der Verwandtschaftsgrade unter den Eltern der Eigenschaftsträger nachweisen. Der letztere

nannte Weg wurde in gewissem Ausmasse benutzt, dürfte aber noch mehr ausgenützt werden. Eine Zunahme bei Verwandten kann nämlich mit Milieumomenten zusammenhängen. Dass tuberkulöse Eltern Kinder haben, die an Tuberkulose leiden, kann ja z. B. auf Ansteckung beruhen. Hingegen kann eine eventuelle Erhöhung der Frequenz an Verwandtschaftsgraden bei den Eltern tuberkulöser Personen nicht auf etwas anderem beruhen als eine Selektive Auswahl durch Erbfaktoren, weswegen eine solche Erhöhung eine grössere Beweiskraft besitzt.

Es ist von Interesse zu versuchen eine Vorstellung darüber zu erhalten, welche Erhöhung man bei polyhybrider Vererbung erwarten kann. Wir nehmen an, dass eine rezessive polyhybride Eigenschaft aus den Anlagen  $R_1 R_1, R_2 R_2 \dots R_n R_n$  mit den Frequenzen  $r_1^2, r_2^2 \dots r_n^2$  zusammengesetzt ist und dass ferner für jedes Anlagepaar eine Vetternehe eine Erhöhung von  $\delta_1, \delta_2 \dots \delta_n$  verursacht. Bei Panmixie würde man erwarten, dass die Eigenschaftsträger die Frequenz  $r_1^2, r_2^2 \dots r_n^2$  haben. Auf Grund der Verwandtschaftsgraden erhält man die Frequenzen  $(r_1^2 + \delta_1), (r_2^2 + \delta_2) \dots (r_n^2 + \delta_n)$ . Nehmen wir ferner an, dass Verwandtschaftsgraden bei Panmixie mit einer Frequenz von  $c_1$  zu erwarten sind und in der Population mit der Frequenz  $c_2$  vorkommen. so wird der Anteil an Eigenschaftsträgern,  $k$ , der von Vetternehen stammt, betragen:

$$k = \frac{c (r_1^2 + \delta_1) (r_2^2 + \delta_2) \dots (r_n^2 + \delta_n)}{(1 - c_1 + c_2) r_1^2 r_2^2 \dots r_n^2 + (c_1 - c_2) (r_1^2 + \delta_1) (r_2^2 + \delta_2) \dots (r_n^2 + \delta_n)}$$

Liegt z. B. eine Dihybridität vor und ist  $r_1 = r_2 = r$ , so erhält die Formel folgendes Aussehen:

$$k = \frac{c}{c_1 - c_2 + \frac{(1 - c)^2 256 r^2}{(1 + 15 r)^2}} \quad (46)$$

Es muss daran erinnert werden, dass die Eigenschaftsträger in diesem Falle die Frequenz  $r^4$  haben. Aus den Formeln ergibt sich, dass die Erhöhung, welche auf Grund dessen, dass eventuell Vetternehen häufiger vorkommen, als bei Panmixie bei einer bestimmten Frequenz der Eigenschaftsträger zu erwarten ist, bei dihybrider oder polyhybrider Rezessivität niedriger ist als bei monohybrider Rezessivität. Es ergibt sich ferner, dass der Anteil an Eigenschafts-

trägern der aus Vetternehen stammt, bei Polyhybridität ebenfalls geringer ist als bei Monohybridität. In diesem Zusammenhange soll daran erinnert werden, dass HALDANE gezeigt hat (1938), dass falls eine monohybride Triallelie vorliegt und ein Gen in homozygoter Form ein Letalfaktor ist, so erhält man einen niedrigeren Anteil an Eigenschaftsträgern aus Vetternehen und eine geringere Erhöhung an Vetternehen, als man bei monohybrider Triallelie erwarten würde. Ein solcher Mechanismus ist indessen in menschlichen Populationen noch nicht festgestellt worden.

Schliesslich soll erwähnt werden, dass die hier angegebenen Formeln selbstverständlich angewandt werden können, um die Frequenz der Anlagen zu berechnen, falls man den Vererbungsmechanismus und die Frequenz an Vetternehen unter den Eltern der Eigenschaftsträger kennt. Sind zum Beispiel 15 % der Eigenschaftsträger Vettern und ist die allgemeine Frequenz an Vetternehen 2 %, so wird die Anlage die Frequenz 1 : 105 besitzen; ist die allgemeine Frequenz an Vetternehen 1 %, so wird die Anlage die Frequenz 1 : 225 oder 0.44 % haben. Ist die allgemeine Frequenz an Vetternehen 0.5 %, so beträgt der entsprechende Wert 1 : 465 oder 0.22 %. Diese Werte werden wir im folgenden eingehender besprechen. Selbstverständlich kann man auch, falls man die Vererbungsart, die Frequenz der Eigenschaftsträger und den Anteil an Eigenschaftsträgern, der von Vetternehen her stammt kennt, die Frequenz an Vetternehen in der Population berechnen.

Eine Ursache dafür, dass man die Frequenz an Verwandtschaftsehen nicht in so grossem Ausmasse untersucht und zu Untersuchungen über Erbllichkeit benutzt hat, ist die Schwierigkeit, Normalwerte zu erhalten. Aus einigen Ländern können offizielle Werte erhalten werden (vgl. DAHLBERG 1938). Diese Werte können ja nicht ohne weiteres zum Vergleich mit einem Material angewandt werden, das aus anderen Populationen stammt, wenn man auch der Ansicht sein kann, dass sie einen ungefähren Ausgangspunkt darstellen. Man dürfte davon ausgehen können, dass die Frequenz an Verwandtschaftsehen selten 1 % übersteigt und sich gegenwärtig wahrscheinlich unter  $\frac{1}{2}$  % hält. Wir werden auf diese Frage später zurückkommen.

In diesem Zusammenhange soll hervorgehoben werden, dass Werte, die erhalten werden, falls man von Patienten ausgeht, die in ein Krankenhaus aufgenommen sind, für die Verhältnisse in einer Popu-

lation nicht repräsentativ sind. Unter den Patienten sind selbstverständlich einige erblich bedingte Krankheitsfälle vorhanden, deren Eltern eine wesentlich erhöhte Frequenz an Verwandtschaftsehen aufweisen. Ist auch diese Gruppe klein, so beeinflusst sie doch die Durchschnittswerte in erhöhender Richtung. JULIA BELL (1940) gelangte zu einem Wert an Vetternehen von 0.61 % bei Patienten in einigen englischen Krankenhäusern. Dieser Wert erscheint für ein Land wie England ungewöhnlich hoch zu sein, da ja in diesem die Kommunikationen gut und Isolate verhältnismässig gross sind. Nimmt man an, dass der wirkliche Wert für die Population 0.25 % ist und dass im Material einige Personen enthalten sind, die an seltenen, erblichen Krankheiten leiden und deren Eltern z. B. in 10 % Vettern und Basen sind, so kann man berechnen, dass diese Gruppe 3.7 % des Materials ausmachen muss. Es ist an und für sich nicht unangebracht anzunehmen, dass die Summe seltener erblich bedingter Krankheiten ein oder zwei Prozent beträgt, d. h. dass Kinder aus Vetternehen überrepräsentiert sind (längerer Krankenhausaufenthalt) und dass dies bedingt, dass das Material als Unterlage für eine Beurteilung der Verhältnisse draussen in der Bevölkerung von zweifelhaftem Wert ist.

### Koppelung und Verwandtschaftsehe.

Bei der Verteilung der Eigenschaften in einer Population bei Panmixie muss man erwarten, dass sie in einem gewissen Umfang zufallsbedingt bei ein und derselben Person zusammentreffen werden. Liegt eine Koppelung vor, müssen sie öfters zusammentreffen. Es muss mit anderen Worten zwischen dem Vorkommen bestimmter erblich bedingter Eigenschaften eine Korrelation vorliegen. Man hat in den letzten Jahren auf dem Gebiete der Erbllichkeitsforschung ein grosses Interesse für die Koppelung beim Menschen gezeigt und es wurden spezielle Methoden für die Feststellung einer Koppelung bei der Untersuchung von Familien und Geschlechter ausgearbeitet.

In der vorliegenden Arbeit sind ja die Fragestellungen auf die Beschaffenheit ganzer Populationen begrenzt. Im Hinblick auf die Koppelung kann man sich damit begnügen festzustellen, dass Korrelationen zwischen dem Auftreten von Eigenschaften ihre Ursache in einer Koppelung haben können. Solche Korrelationen können

indessen auch andere Ursachen haben. Wir werden auf dieses Problem zurückkommen. In diesem Zusammenhange soll nur die Korrelation behandelt werden, die durch Verwandtschaftsehe verursacht werden kann.

Will man untersuchen, wie die Eigenschaften in einer Population zusammentreffen, so muss man auch berücksichtigen, dass die Population in zwei Gruppen zerfällt: Nachkommen verwandter Personen und andere. Wir begnügen uns damit in diesem Zusammenhange nur mit Vetternehen zu rechnen und von anderen Verwandtschaftsehen abzusehen, da wie vorher hervorgehoben, Verwandtschaftsehen mit stärkerer Wirkung äusserst selten sind und abgelegene Verwandtschaftsehen eine wesentlich schwächere Erhöhung der Homozygotie verursachen. Rezessive Eigenschaften haben nun wahrscheinlich in diesen Teilen der Population eine verschiedene Frequenz. Unter den Nachkommen aus Vetternehen ist die Frequenz höher, wenn es sich um seltene Eigenschaften handelt als unter Nachkommen aus den übrigen Ehen. Die Wahrscheinlichkeit dafür, dass zwei seltene Eigenschaften zusammentreffen werden, ist also unter den Nachkommen von Vettern grösser als unter den Nachkommen von Personen, die nicht Vetter und Base sind. Berechnet man nun wie oft Eigenschaftsträger zusammentreffen müssen und nimmt als Grundlage hierzu die Frequenz, welche sie in der Population haben, und untersucht wie oft dies faktisch geschieht, so findet man eine Erhöhung, die eine Korrelation zwischen den Eigenschaften darstellt.

Nehmen wir an, dass in der Population Vetternehen mit der Frequenz  $c$  vorkommen und dass also andere Ehen die Frequenz  $1-c$  aufweisen, ferner, dass zwei rezessive Eigenschaften die Frequenz  $p_1$  und  $p_2$  haben. Schliesslich nehmen wir an, dass  $k_1$  der Eigenschaftsträger aus Vetternehen stammen, und zwar bezüglich der Eigenschaft  $p_1$  und  $k_2$  für die Eigenschaft  $p_2$ . Die Frequenz der Eigenschaftsträger unter den Nachkommen aus Vetternehen ist

$$\frac{p_1 k_1}{c} \text{ bzw. } \frac{p_2 k_2}{c}$$

und unter dem übrigen Teil der Bevölkerung

$$\frac{p_1 (1 - k_1)}{1 - c} \text{ und } \frac{p_2 (1 - k_2)}{1 - c}$$

Zufallsbedingt erwartet man, dass die Eigenschaftsträger in der Frequenz  $p_1 p_2$  zusammentreffen. In der Wirklichkeit treffen sie in folgender Frequenz zusammen:

$$\frac{p_1 p_2 (1 - k_1) (1 - k_2)}{1 - c} + \frac{p_1 p_2 k_1 k_2}{c}$$

Das Verhältnis zwischen wirklicher und erwarteter Frequenz ist also:

$$y = \frac{(1 - k_1) (1 - k_2)}{1 - c} + \frac{k_1 k_2}{c} \quad (47)$$

TABELLE 7.

Zusammentreffen zweier rezessiver, monohybrider, dialleler Eigenschaften ( $y$ ) mit gleicher Frequenz bei verschiedener Häufigkeit von Geschwisterkinderehen ( $c$ ), wenn der Anteil der Eigenschaftsträger, der von Geschwisterkinderehen herkommt verschiedene Grösse ( $k$ ) hat. (Dies beruht darauf, dass die Eigenschaftsträger variierende Frequenz haben.) Vgl. Formel 47.

$k$ , %	$y$ für			
	$c = 0.001$	$c = 0.0025$	$c = 0.005$	$c = 0.01$
1	1.081	1.023	1.005	1.000
2	1.301	1.123	1.045	1.010
3	1.842	1.303	1.120	1.040
4	2.523	1.564	1.246	1.091
5	3.403	1.905	1.407	1.162
10	10.811	4.812	2.814	1.818
15	23.223	9.724	5.226	2.980
30	90.491	36.491	18.493	9.455

Aus Tabelle 7 und Abb. 15 wird eine Orientierung über die Bedeutung von Verwandtschaftsehen für das Zusammentreffen von Eigenschaften erhalten. Die Werte wurden unter der Annahme berechnet, dass  $p_1 = p_2$  und also  $k_1 = k_2$ . Die Wirkung ist um so stärker je geringer die Frequenz an Vetternehen ist und je grösser der Anteil an Eigenschaftsträgern ist, die aus Vetternehen stammen. Dieser Anteil ist, wie bereits gezeigt wurde, um so grösser, je seltener die Eigenschaft ist und je grösser die Frequenz der Vetternehen ist. Durch Anwendung der Werte in Tabelle 6 und Tabelle 7 erhält man eine Orientierung. Geht man z. B. davon aus, dass Vetternehen die Frequenz von 0.25 % haben, ein Wert, der als angebracht ange-

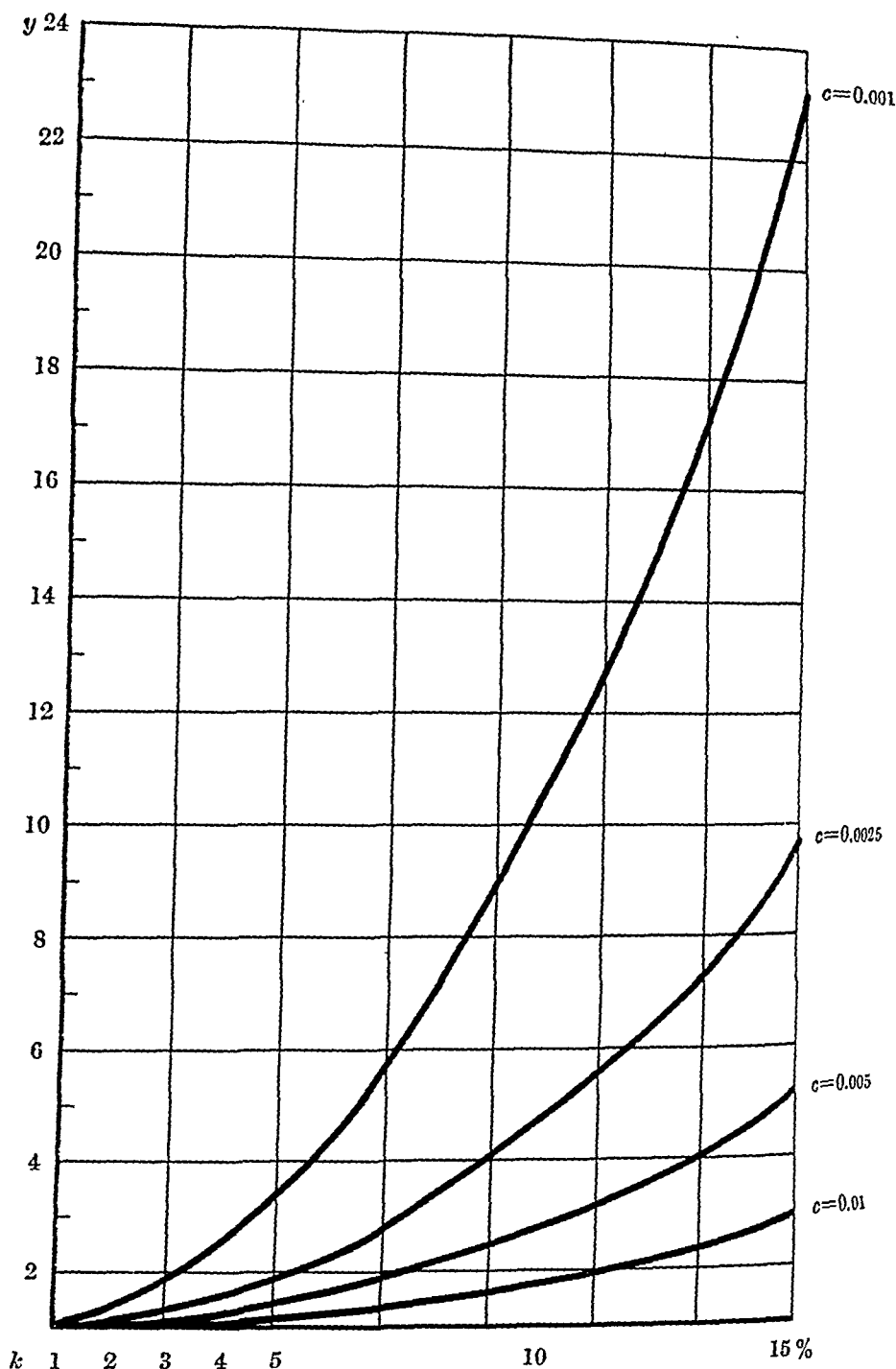


Abb. 15. Zusammentreffen zweier rezessiver Eigenschaften ( $y$ ) mit gleicher Frequenz in der Bevölkerung, wenn Geschwisterkinderehen verschiedene Frequenz haben und der Anteil Eigenschaftsträger, der von Geschwisterkinderehen her stammt, verschiedene Grösse ( $k$ ) hat. (Vgl. Tabelle 7.)

sehen werden kann, so findet man eine feststellbare Wirkung in Form einer erhöhten Korrelation, da über 2 % der Eigenschaftsträger aus Vetternehen stammen, was eine Eigenschaftsfrequenz von etwas weniger als 1 : 10 000 bedeutet. Haben die Vetternehen eine Frequenz von 0.5 %, so erhält man eine feststellbare Einwirkung bei einer Frequenz der Eigenschaftsträger von etwas mehr als 1 : 10 000. Durch Anwendung der Formeln 46 und 47 kann man schliesslich auch das Verhältnis zwischen wirklicher und der bei Panmixie zu erwartenden Frequenz für das Zusammentreffen von Eigenschaften berechnen, wenn die jeweilige Anlage die Frequenz  $r_1$  und  $r_2$  hat und wenn die Frequenz für Vetternehen  $c$  ist und der bei Panmixie zu erwartenden entspricht.

Die Formel wird dann:

$$y = \frac{\left(1 - \frac{c(1 + 15r_1)}{16r_1}\right) \left(1 - \frac{c(1 + 15r_2)}{16r_2}\right)}{1 - c} + \frac{c(1 + 15r_1)(1 + 15r_2)}{256r_1r_2} \quad (48)$$

TABELLE 8.

Zusammentreffen zweier rezessiver, monohybrider, dialleler Eigenschaften ( $y$ ) mit gleicher Frequenz bei verschiedener Frequenz der Geschwisterkindernehen ( $c$ ), wenn die Eigenschaftsträger variierende Frequenz haben ( $RR$ ). Vgl. Formel 48.

RR	$y$ für							
	$c = 0.001$		$c = 0.0025$		$c = 0.005$		$c = 0.01$	
0.000001	$k, 6.3$	4.002	$k, 15.9$	10.771	$k, 31.7$	20.501	$k, 63.4$	40.378
0.00005	% 1.0	1.077	% 2.4	1.193	% 4.9	1.387	% 9.8	1.778
0.0001	0.7	1.038	1.8	1.096	3.6	1.102	7.2	1.387
0.0005	0.4	1.008	0.9	1.019	1.9	1.038	3.7	1.075
0.001	0.3	1.004	0.7	1.009	1.5	1.018	2.9	1.037

Mit Hilfe dieser Formel sind in Tabelle 8 einige Werte berechnet worden, ausgehend davon, dass  $r_1 = r_2 = r$ . Aus der Tabelle ergibt sich, dass die Quote den Wert 1 erst dann deutlich überschreitet, wenn die Frequenz der Eigenschaftsträger 1 auf 10 000 oder niedriger ist. Bei gewisser Frequenz einer Anlage ist die Verschiebung



stärker, je höher die Frequenz von Vetternehen ist. Es ist deutlich, dass wir eine Korrelation zwischen Eigenschaften vor allem dann zu erwarten haben, wenn es sich um seltene Anlagen handelt und bei sehr seltenen Eigenschaften können wir eine sehr kräftige Verschiebung erhalten. Aus den Berechnungen ergibt sich mit anderen Worten, dass man erst bei sehr seltenen Anlagen ein bemerkbar häufigeres Zusammentreffen von Eigenschaften bei ein und derselben Person auf Grund von Vetternehen feststellen kann. Der Prozess ist aber für häufig vorkommende Eigenschaften ohne Bedeutung.

Es muss aber daran erinnert werden, dass bei seltenen Eigenschaften der Prozess auf alle Eigenschaften wirkt, die rezessiv oder auf eine kompliziertere Art vererbt werden. In Wirklichkeit kann ein Prozess dieser Art die Erklärung dafür sein, dass Missbildungen verschiedener Art verhältnismässig oft zusammentreffen. Einige Missbildungen beruhen ja auf intrauterinen Milieufaktoren, die gleichzeitig Schädigungen mehrerer Organe bewirken. In der Literatur über erbliche Missbildungen findet man aber Angaben über das Zusammentreffen klar erblich bedingter Missbildungen, z. B. Syndaktylie, Polydaktylie Palatoschisis, kongenitales Vitium u. s. w. Ein derartiges Zusammentreffen kann Ausdruck einer Koppelung sein. Der variierende Charakter der erblichen Missbildungen, die zusammentreffen, deutet aber am ehesten darauf, dass Vetternehen eine entscheidende Rolle spielen. Die Probleme wurden aber nicht Gegenstand einer näheren Untersuchung aus den Gesichtspunkten, die hier angelegt wurden, weswegen keine endgültigen Feststellungen gemacht werden können.

Selbstverständlich haben Verwandtschaftsehen auch für das Auftreten von Eigenschaften bei Geschwistern der Eigenschaftsträger Bedeutung. Unter Kindern aus Vetternehen liegt eine Homozygotie für seltene erbliche Eigenschaften vor. Wählt man Eigenschaftsträger aus, die in besonderer Ausdehnung von Vetternehen herkommen, so hat man deswegen zu erwarten, dass ihre Geschwister eine erhöhte Frequenz anderer seltener rezessiver Eigenschaften aufweisen werden. Man kann deswegen eine solche Korrelation zwischen Geschwistern nicht ohne weiteres als Ausdruck für eine Koppelung deuten. LUXENBURGER (1927) hat z. B. versucht zu zeigen, dass unter den Verwandten gewisser Geisteskranker (Schizophrenie) Tuberkulose in grösserem Ausmasse vorkommt, als man zufallsbedingt zu erwarten hat. Seine Untersuchungen wurden einer scharfen Kri-

tik ausgesetzt (ALSTRÖM, 1942). Das Problem ist aber nicht völlig aufgeklärt, auch wenn die Werte, die LUXENBURGER angeführt hat, keine Beweiskraft besitzen. Würden erbliche Momente sowohl für Tuberkulose als auch für Schizophrenie eine Rolle spielen, so können Vetternehen eine Korrelation zwischen den Eigenschaften in Geschwisterkreisen verursachen, falls die Anlagen ausreichend selten sind. Dieser Mechanismus, der bisher nicht beachtet wurde, kann auch von Bedeutung sein, wenn man eine Koppelung durch Familienuntersuchungen nachweisen will, aber wie oben hervorgehoben, fallen diese Probleme ausserhalb des Rahmens dieser Arbeit.

*Zusammenfassend* soll hervorgehoben werden, dass die Verwandtschaftsehe für gewöhnliche Eigenschaften ohne Bedeutung ist. Für seltene rezessive und für auf kompliziertem Wege erbliche Eigenschaften spielt die Verwandtschaftsehe eine grössere Rolle und bei extremen Fällen, wenn eine Anlage durch Mutation das erste Mal entsteht, kann diese in homozygoter Form nur durch Eheschliessung zwischen Nachkommen des Mutanden, d. h. durch Verwandtschaftsehe, zusammentreffen. Ist eine Eigenschaft selten, so ist sie indessen für die Zusammensetzung der Population ohne nennenswerte Bedeutung. Die Wirkung der Verwandtschaftsehe steht also mit der Frequenz der Anlage im Gleichgewicht, so dass die Verwandtschaftsehe nie mitsichführt, dass *eine* bestimmte Eigenschaft für eine Population eine nennenswerte Bedeutung erhält. Während die Selektion auf gewöhnliche Eigenschaften eine bedeutende Wirkung ausübt, für sehr seltene Eigenschaften aber ohne nennenswerte Bedeutung ist, ist die Situation für die Verwandtschaftsehe die umgekehrte, da diese für gewöhnliche Eigenschaften bedeutungslos ist, für seltene Eigenschaften aber *vom Gesichtspunkt des Eigenschaftsträgers* aus grosse Bedeutung besitzt. Während die Selektion nur auf einzelne seltene Eigenschaften einwirkt, führt eine Änderung der Frequenz von Verwandtschaftsehen eine Änderung der Häufigkeit *aller* seltener Eigenschaften mit sich. Die gesamte Wirkung kann vom Gesichtspunkt der Gesellschaft aus eine Rolle spielen. Diese Gesichtspunkte sollen im Zusammenhang mit der Besprechung der Wirkung der Verwandtschaftsehe und der Isolatgrenzen weiter unten ausführlicher behandelt werden. Auf Grund dessen, dass die Verwandtschaftsehe die Frequenz der seltenen Eigenschaften beeinflusst, ist zu erwarten, dass die Eltern seltener Eigenschaftsträger in verhältnismässig grosser Ausdehnung verwandt

(Vetter und Base) sein müssen. Umgekehrt hat man auch eine Korrelation zwischen dem Vorkommen seltener Eigenschaften zu erwarten, da man bei der Auswahl von solchen von Personen ausgeht, die in verhältnismässig grosser Ausdehnung miteinander verwandt (Vetter und Base) sind und bei deren Nachkommenschaft man eine Anhäufung von seltenen Eigenschaftsträgern zu erwarten hat.

## Verwandtschaftsehe und Selektion.

Früher wurde hervorgehoben, dass man bei Panmixie eine bestimmte Frequenz von Verwandtschaftsehen zu erwarten hat. Wir haben ferner Formeln für die Wirkung der Selektion bei Panmixie angegeben. Die Frage ist nun, welche Wirkung eine höhere oder geringere Frequenz von Verwandtschaftsehen als die bei Panmixie zu erwartende, auf einen Selektionsprozess ausübt. Beide Möglichkeiten liegen in der menschlichen Bevölkerung insofern vor, als bei einem Teil der Bevölkerung die Frequenz an Verwandtschaftsehen niedriger ist als man bei Panmixie zu erwarten hat, und zwar deswegen, weil Vetternehen verboten sind. Eine etwas höhere Frequenz von Vetternehen als die, welche der Panmixie entspricht, liegt wahrscheinlich in Bevölkerungen vor, in welchen die Ehen durch die Eltern beider Kontrahenten geordnet werden. Beide Fragestellungen besitzen mit anderen Worten praktisches Interesse.

Wir fragen uns also in erster Linie, welche Bedeutung möglicherweise das Ausbleiben von Vetternehen haben kann. Werden Vetternehen verhindert und würden solche bei Panmixie die Frequenz  $c$  haben, so würde die Bevölkerung folgende Zusammensetzung erhalten:

$$r^2 - c \left( \frac{r}{16} + \frac{15r^2}{16} \right)$$

Der Anteil, der wegfällt, ist also:

$$c \left( \frac{1}{16} r + \frac{15}{16} r^2 \right) \quad (49)$$

oder in Verhältnis zu  $r^2$

$$c \left( \frac{1}{16r} + \frac{15}{16} \right)$$

Findet eine Zunahme der Vetternehen über das hinaus statt, was bei Panmixie mit einer Frequenz von  $c$  zu erwarten war, so gelten

dieselben Formeln, aber mit umgekehrtem Vorzeichen vor  $c$ . Die Zunahme bzw. Abnahme der Eigenschaftsträger, die man bei einigen verschiedenen Frequenzen von  $c$  und einigen Frequenzen von  $r^2$  erhält, sind mit Hilfe der Formel leicht zu berechnen.

Man muss sich daran erinnern, dass eine Abnahme, verglichen mit Panmixie, nicht grösser sein kann, als die Frequenz von Verwandtschaftsehen, die man bei Panmixie zu erwarten hat, während es aber für eine Zunahme theoretisch ein Spielraum bis zu 100 % vorliegt.

Aus den Ausdrücken in der Tabelle ergibt sich, dass übereinstimmend mit dem, was vorher gezeigt wurde, die Verwandtschaftsehe erst dann für die Frequenz der Eigenschaftsträger Bedeutung besitzt, wenn die Anlage in der Bevölkerung selten ist, und dass die Wirkung früher eintritt, wenn es sich um eine grössere Veränderung der Frequenz von Vetternehen verglichen mit Panmixie handelt als wenn die Veränderung gering ist. Prinzipiell gesehen bedeutet dies, dass die Wirkung der Selektion bei selteneren Eigenschaften grösser wird, wenn Verwandtschaftsehen in grösserem Ausmasse als bei Panmixie vorkommen. Die Erhöhung, welche durch Verwandtschaftsehen erhalten wird, ist aber insoferne nicht von wesentlicher Bedeutung, da bei einer so starken Zunahme (oder Abnahme) von Verwandtschaftsehen wie 1 %, erst wenn die Eigenschaftsträger eine Frequenz von 1 : 1 000 000 haben, eine Zunahme (bzw. Abnahme) der Eigenschaftsträger um 63 %, d. h. eine Zunahme, bzw. Abnahme um etwas mehr als die Hälfte, erhalten wird. Bei einer bestimmten Mutationsfrequenz werden die Abweichungen in der Frequenz der Verwandtschaftsehen eine gewisse Rolle für die Gleichgewichtslage, die erreicht wird, spielen. Die Heterozygotenfrequenz, welche der Gleichgewichtslage entspricht, wird etwas grösser, wenn Verwandtschaftsehen in geringerem Ausmasse vorkommen, als man sie bei Panmixie zu erwarten hat und etwas geringer im entgegengesetzten Fall. Die Verschiebung dürfte indessen in menschlichen Populationen keine grössere Bedeutung besitzen. Wir werden auf dieses Problem im Zusammenhang mit der Frage nach der Bedeutung der Isolate zurückkommen.

## Gattenwahl.

Mit Gattenwahl („assortative mating“) bezeichnet man das Verhalten, dass Kreuzungen zwischen Personen, die gewisse Eigenschaften aufweisen, in grösserem Ausmasse stattfinden, als man es zufallsbedingt zu erwarten hat, und dass also Kreuzungen entgegengesetzter Art in entsprechendem Ausmasse an Frequenz abgenommen haben. Eingehendere Untersuchungen darüber, in welchem Ausmasse eine Gattenwahl bei Menschen vorkommt, wurden nicht durchgeführt. Es wurde aber festgestellt, dass Personen, die gross bzw. klein sind, sich öfter mit Personen derselben Körperlänge verheiraten, als was zufallsbedingt zu erwarten ist, was selbstverständlich dadurch bedingt wird, dass grosse und kleine Personen in einem etwas komischen Kontrast zueinander stehen (PEARSON and LEE 1903). Es liegt mit anderen Worten ein gewisser Widerstand gegen die Ehe zwischen grossen und kleinen Personen vor. Man hat ferner Anlass zu behaupten, dass Taubstumme sich verhältnismässig oft miteinander verheiraten. Das Leiden erschwert den Kontakt mit gesunden Personen, ist aber ein verbindendes Band zwischen den Betroffenen. Man kann vermuten, dass musikalische Personen sich besonders oft miteinander verheiraten, sowie dass überhaupt erbliche Eigenschaften, die eine spezielle Einstellung von Interessen verursachen, einen gewissen Grad von Gattenwahl bedingen; Interessen vereinen. Inwieweit man so weit gehen und behaupten kann, dass intelligente Personen sich öfter miteinander verheiraten, als es der Zufall bedingt, ist unsicherer.

Man hat aber damit zu rechnen, dass nicht nur eine positive Gattenwahl vorkommt, bei der sich Personen mit denselben Eigenschaften miteinander verheiraten. Die Möglichkeit einer negativen Gattenwahl, wobei ein Widerstand gegen die Verheiratung zwischen den Personen vorliegt, welche die gleiche Eigenschaft haben, ist ebenfalls vorstellbar. Es ist z. B. möglich, dass extrem eigensinnige oder temperamentvolle Personen verhältnismässig leicht in Konflikt geraten, und dass in dem Masse, in welchem solche Eigen-

schaften erblich bedingt sind, eine negative Gattenwahl vorkommt. Wenn es sich um Eigenschaften handelt, die eine gewisse Hilflosigkeit bedingen (erbliche Blindheit, Verkrüppelung), kann die Hilflosigkeit selbst ein Hindernis für die Ehe zwischen Personen, die am gleichen Gebrechen leiden, mitführen, während dagegen eine Ehe mit normalen nicht in gleichem Ausmass unmöglich erscheint. Direkte Untersuchungen, welche das Vorkommen einer negativen Gattenwahl nachweisen, wurden indessen bisher nicht ausgeführt. Auf Grund dessen, dass man sich bisher für Verschiebungen in der Erbmasse einer Bevölkerung nicht allgemeiner interessiert hat, ist die empirische Unterlage für Prozesse dieser Art sehr mangelhaft.

Man kann nun ferner zwischen totaler Gattenwahl, bei welcher die Eigenschaftsträger sich ausschliesslich miteinander verheiraten und keiner, der die Eigenschaft besitzt, sich mit einem anderen, dem sie fehlt, kreuzt, und partieller Gattenwahl unterscheiden, bei welchen die Eigenschaftsträger sich nur etwas häufiger miteinander verheiraten als man es bei Panmixie zu erwarten hat. Die Wirkung einer totalen positiven Gattenwahl wurde von ROBINS (1918) berechnet. Für die partielle positive Gattenwahl wurden Rekursionsformeln von KOLLER (1938) angegeben, der auch mit Hilfe derselben Grenzwerte mitgeteilt hat. Beide Autoren wenden eine kompliziertere Bezeichnungsart an. KOLLER hat die Annahme benutzt, dass ein bestimmter Anteil von Verbindungen zwischen dominanten und rezessiven Eigenschaftsträgern ausbleibt. Im Folgenden werden Formeln abgeleitet, die eine einfachere Form haben, ausserdem wird auch ein neuer Ausgangspunkt für die Lösung des Problems angegeben, der auch eine allgemeinere Formel erlaubt.

Der Effekt negativer Gattenwahl ist nicht früher analysiert worden.

### Positive totale Gattenwahl.

Bei positiver totaler Gattenwahl und monohybrider Diallelie werden gemäss früherer angewandter Bezeichnungen Personen, welche die Beschaffenheit  $RR$  haben, sich immer miteinander verheiraten und die übrigen Typen sich zufallsbedingt miteinander verheiraten. In der ersten Generation erhalten wir dann untenstehende Kreuzungen und Nachkommen aus diesen Kreuzungen. Vgl. Tabelle 9.

TABELLE 9.

Vgl. Text.

Elternkombinationen	Häufigkeit in der Population	Kinder		
		Häufigkeit der Typen		
		RR	RD	DD
RR × RR	$r^4 + 2 r^3 d + r^2 d^2 = r^3 = \alpha$	$\alpha$		
RR × RD	$4 r^3 d - 4 r^3 d = 0$	0	0	
RR × DD	$2 r^2 d^2 - 2 r^2 d^2 = 0$		0	
RD × RD	$4 r^2 d^2 + \frac{4 r^2 d^2 (2 r^2 d + r^2 d^2)}{(2 r d + d^2)^2} = \frac{4 r^2 d}{1 + r} = d$	$\frac{1}{4} d$	$\frac{1}{2} d$	$\frac{1}{4} d$
RD × DD	$4 r d^3 + \frac{4 r d^3 (2 r^2 d + r^2 d^2)}{(2 r d + d^2)^2} = \frac{4 r d^3}{1 + r} = c$		$\frac{1}{2} c$	$\frac{1}{2} c$
DD × DD	$d^4 + \frac{d^4 (2 r^2 d + r^2 d^2)}{(2 r d + d^2)^2} = \frac{d^3}{1 + r} = f$			f
Summen		$RR = \alpha + \frac{1}{4} d = r^3 + \frac{r^3 d}{1 + r}$	$RD = \frac{1}{2} d + \frac{1}{2} c = 2 r d - \frac{2 r^3 d}{1 + r}$	$DD = \frac{1}{4} d + \frac{1}{2} c + \frac{r^2 d}{1 + r}$ $+ f = d^3 + \frac{r^2 d}{1 + r}$



Setzt man auf diese Weise fort, so findet man nach  $n$  Generationen

$$r_n^2 = r^2 + \frac{nr^2 d}{1 + nr} = \frac{(n+1)r^2}{1 + nr} \quad (50)$$

Eigenschaftsträger vom Typus  $RR$

$$d_n^2 = d^2 + \frac{nr^2 d}{1 + nr} \quad (51)$$

vom Typus  $DD$  und

$$2r_n d_n = 2rd - \frac{2nr^2 d}{1 + nr} \quad (52)$$

vom Typus  $RD$ .

In der nächsten Generation, d. h. der Generation  $n+1$  erhalten wir dann folgende Wahrscheinlichkeit für die Eigenschaftsträger vom Typus  $RR$

$$r_{n+1}^2 = \frac{(n+2)r^2}{1 + (n+1)r}$$

Dass diese Formel gilt, ist aus Folgendem zu sehen. In der  $n$ . Generation ist das Verhältnis der rezessiven Anlage unter den Heterozygoten und den dominanten Homozygoten in der Bevölkerung

$$\frac{\left[ r - \frac{(n+1)r^2}{1 + nr} \right]^2}{1 - \frac{(n+1)r^2}{1 + nr}}$$

Die Eigenschaftsträger in der  $n$ . Generation verheiraten sich ausschliesslich miteinander und ergeben in der  $(n+1)$ . Generation dieselbe Proportion an Eigenschaftsträgern, wie sie selbst in der  $n$ . Generation ausmachten. Zu diesen Eigenschaftsträgern kommen in dessen Eigenschaftsträger, die aus zufallsbedingten Kreuzungen zwischen Nicht-Eigenschaftsträgern entstanden sind. Aus diesen letzteren Kreuzungen wird eine Anzahl von Eigenschaftsträgern erhalten, die durch die untenstehende Formel angegeben wird.

$$\frac{\left[ r - \frac{(n+1)r^2}{1 + nr} \right]^2}{1 - \frac{(n+1)r^2}{1 + nr}} = \frac{r^2(1-r)}{[1 + nr - (n+1)r^2][1 + nr]}$$

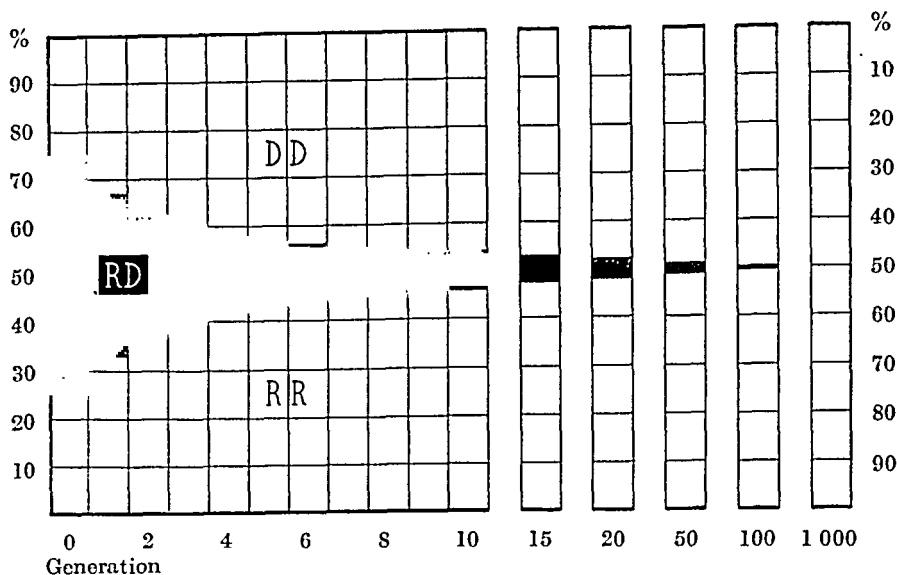


Abb. 16. Frequenz von Heterozygoten bei totaler Gattenwahl in sukzessiven Generationen, wenn eine rezessive Anlage die Frequenz  $r = 0.5$  hat. (Vgl. Tabelle 10.)

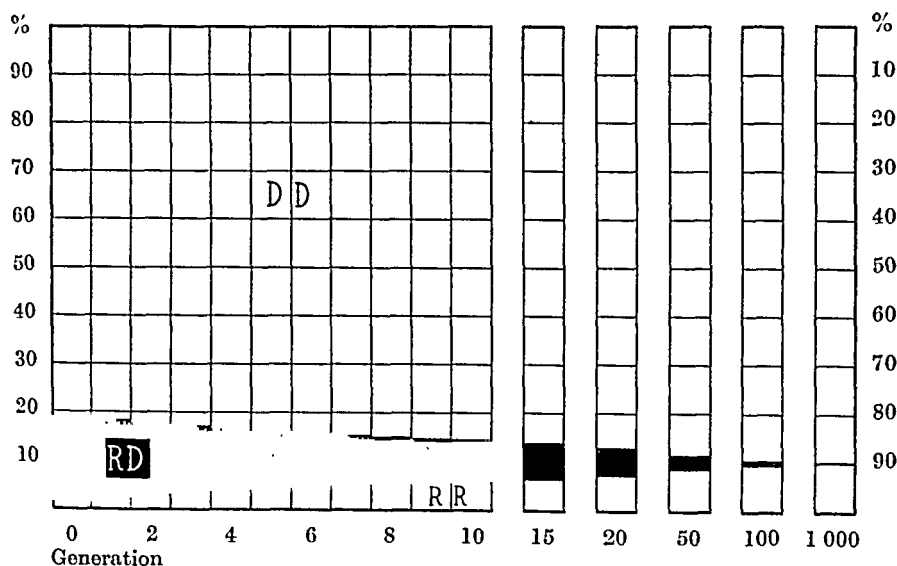


Abb. 17. Frequenz von Heterozygoten bei totaler Gattenwahl in sukzessiven Generationen, wenn eine rezessive Anlage die Frequenz  $r = 0.1$  hat. (Vgl. Tabelle 10.)

Insgesamt erhalten wir also in der  $(n + 1)$ . Generation die folgende Anzahl von Eigenschaftsträgern

$$r_{n+1}^2 = \frac{(n+1)r^2}{1+nr} + \frac{r^2(1-r)}{[1+nr - (n+1)r^2][1+nr]} = \frac{(n+2)r^2}{1+(n+1)r}$$

Hieraus ergibt sich, dass falls die Formel für  $n$  Generationen gilt, sie auch für  $(n + 1)$  Generationen Gültigkeit besitzt. Es wurde schon gezeigt, dass die Formel für die 1. Generation gilt.

Der Grenzwert wird durch Einsetzen von  $n = \infty$  erhalten. Man findet dann, dass die Heterozygoten verschwinden und dass die Population aus Personen vom Typus  $RR$  mit der Frequenz  $r$  und Personen vom Typus  $DD$  und der Frequenz  $d$  bestehen wird. Man erhält eine Vorstellung darüber wie schnell der Prozess vor sich geht durch Abb. 16 und 17 und Tabelle 10.

TABELLE 10.

Effekt der positiven, totalen Gattenwahl in aufeinanderfolgenden Generationen bei monohybrider, rezessiver Diallelie, wenn die Eigenschaftsträger variierende Frequenz haben. Vgl. Formel 50, 51 und 52.

Generation	$r^2 = 0.01\%$			$r^2 = 1\%$			$r^2 = 4\%$			$r^2 = 25\%$		
	RR	RD	DD	RR	RD	DD	RR	RD	DD	RR	RD	DD
0	0.01	1.98	98.01	1.0	18.0	81.0	4.0	32.0	64.0	25.0	50.0	25.0
1	0.02	1.96	98.02	1.8	16.4	81.8	6.7	26.7	66.7	33.3	33.3	33.3
2	0.03	1.94	98.03	2.5	15.0	82.5	8.6	22.9	68.6	37.5	25.0	37.5
3	0.04	1.92	98.04	3.1	13.8	83.1	10.0	20.0	70.0	40.0	20.0	40.0
4	0.05	1.90	98.05	3.6	12.9	83.6	11.1	17.8	71.1	41.7	16.7	41.7
5	0.06	1.88	98.06	4.0	12.0	84.0	12.0	16.0	72.0	42.9	14.3	42.9
6	0.07	1.86	98.07	4.4	11.2	84.4	12.7	14.5	72.7	43.8	12.5	43.8
7	0.07	1.86	98.07	4.7	10.6	84.7	13.3	13.3	73.3	44.4	11.1	44.4
8	0.08	1.84	98.08	5.0	10.0	85.0	13.9	12.3	73.9	45.0	10.0	45.0
9	0.09	1.82	98.09	5.3	9.5	85.3	14.3	11.4	74.3	45.5	9.1	45.5
10	0.10	1.80	98.10	5.5	9.0	85.5	14.7	10.7	74.7	45.8	8.3	45.8
15	0.14	1.72	98.14	6.4	7.2	86.4	16.0	8.0	76.0	47.1	5.9	47.1
20	0.18	1.64	98.18	7.0	6.0	87.0	16.8	6.4	76.8	47.7	4.5	47.7
50	0.34	1.32	98.34	8.5	3.0	88.5	18.6	2.9	78.6	49.0	2.0	49.0
100	0.51	0.98	98.51	9.2	1.6	89.2	19.2	1.5	79.2	49.5	1.0	49.5
1000	0.91	0.18	98.91	9.9	0.2	89.9	19.9	0.2	79.9	50.0	0	50.0
Grenzwert	1.00		99.00	10.0		90.0	20.0		80.0	50.0		50.0

Nun dürfte eine totale Gattenwahl in menschlichen Populationen nicht vorkommen. Das Problem hat für die Tierzucht praktisches Interesse und aus diesem Gesichtspunkt wurde das Problem ursprünglich von ROBINS auf Basis bestimmter Kreuzungsschemata behandelt, die von JENNINGS 1916 und 1917 angegeben wurden.

### Positive partielle Gattenwahl.

Für den Menschen hat man nur mit einer partiellen Gattenwahl zu rechnen, d. h. damit, dass bestimmte Eigenschaftsträger sich miteinander in etwas grösserem Ausmasse als bei Panmixie miteinander kreuzen. Der Prozess muss im Prinzip in derselben Richtung wie eine totale Gattenwahl wirken, d. h. zu einer Zunahme der Homozygoten auf Kosten der Frequenz der Heterozygoten führen. Man fragt sich wie schnell ein solcher Prozess verläuft und gegen welche Grenzwerte. Führt auch dieser Prozess zu einer völligen Homozygotie? Eine Schwierigkeit für die Lösung dieses Problems ist, dass falls man annimmt, dass die Eigenschaftsträger einander in einer bestimmten Frequenz vorziehen, die grösser ist, als die bei Panmixie zu erwartende, so nimmt hierdurch die Frequenz an Homozygoten zu. Wird deren Frequenz höher, ist es indessen schwer zu entscheiden, was eine „unveränderte Tendenz“ in der veränderten Situation bedeuten wird. Da uns empirische Unterlagen für die Annahme fehlen, muss man sich mit Lösungen begnügen, die auf Annahmen aufgebaut sind, die angebracht erscheinen, wenn man auch nicht zu behaupten wagt, dass sie mit Sicherheit mit dem übereinstimmen, was in menschlichen Populationen wirklich stattfindet. Es liegt am nächsten anzunehmen, dass ein konstanter Widerstand gegen die Verbindung von Eigenschaftsträgern vom entgegengesetzten Typus vorliegt. Ein konstantes Verhältnis der Verbindungen zwischen Eigenschaftsträgern und Nicht-Eigenschaftsträgern, das man bei zufallsbedingten Kreuzungen in jeder Population erwarten kann, bleibt aus und die Eigenschaftsträger, die es unterlassen sich miteinander zu verheiraten, verheiraten sich mit Eigenschaftsträgern desselben Typus. Geht man nun von Panmixie aus und nimmt an, dass  $2k$  Ehen von solchen zwischen Eigenschaftsträgern und Nicht-Eigenschaftsträgern ausbleiben, so wird der Prozess zu einer Abnahme der Frequenz der Heterozygoten führen und

TABELLE 11. Vgl. Text.

Eltern-kombina-tionen	Häufigkeit in der Population	Kinder			
		Häufigkeit der Typen			DD <sup>n</sup>
		RR <sup>n</sup>	RD <sup>n</sup>	DD <sup>n</sup>	
RR × RR n-1 n-1	$(r^2 + \Delta)^2 + k 2(r^2 + \Delta)(2rd - 2\Delta) + k 2(r^2 + \Delta)(d^2 + \Delta) = a$	a	$\frac{1}{2}b$	$\frac{1}{4}d$	
RR × RD n-1 n-1	$2(r^2 + \Delta)(2rd - 2\Delta) - 2k 2(r^2 + \Delta)(2rd - 2\Delta) = b$	$\frac{1}{2}b$	c		
RR × DD n-1 n-1	$2(r^2 + \Delta)(d^2 + \Delta) - 2k 2(r^2 + \Delta)(d^2 + \Delta) = c$	$\frac{1}{4}d$	$\frac{1}{2}d$		
RD × RD n-1 n-1	$(2rd - 2\Delta)^2 + \frac{2k[(r^2 + \Delta)(2rd - 2\Delta) + (r^2 + \Delta)(d^2 + \Delta)][2rd - 2\Delta]^2}{[(2rd - 2\Delta) + (d^2 + \Delta)]^2} = d$				
RD × DD n-1 n-1	$2(2rd - 2\Delta)(d^2 + \Delta) + \frac{2k[(r^2 + \Delta)(2rd - 2\Delta) + (r^2 + \Delta)(d^2 + \Delta)][2(2rd - 2\Delta)(d^2 + \Delta)]}{[(2rd - 2\Delta) + (d^2 + \Delta)]^2} = e$		$\frac{1}{2}e$		
DD × DD n-1 n-1	$(d^2 + \Delta)^2 + \frac{2k[(r^2 + \Delta)(2rd - 2\Delta) + (r^2 + \Delta)(d^2 + \Delta)][d^2 + \Delta]^2}{[(2rd - 2\Delta) + (d^2 + \Delta)]^2} = f$			f	
Summen		RR <sup>n</sup> = $a + \frac{1}{2}b + \frac{1}{4}d = r^2 + \frac{2kd^2(r^2 + \Delta)}{1 - (r^2 + \Delta)}$	RD <sup>n</sup> = $\frac{1}{2}b + c + \frac{1}{2}d + \frac{1}{2}e = 2rd - \frac{4kd^2(r^2 + \Delta)}{1 - (r^2 + \Delta)}$	DD <sup>n</sup> = $\frac{1}{4}d + \frac{1}{2}e + f = d^2 + \frac{2kd^2(r^2 + \Delta)}{1 - (r^2 + \Delta)}$	

die Anlagen entgegengesetzter Art, welche daran verhindert werden zusammenzukommen, bilden Homozygoten. Nimmt man an, dass in einer bestimmten Generation die Abnahme der Heterozygoten  $2 \mathcal{A}_n$  ist, so muss jeder der Homozygotentypen  $\mathcal{A}_n$  zugenommen haben. Man kann nun die Verschiebung berechnen, die von dieser Generation zur nächsten stattfindet und also eine Rekursionsformel erhalten, die eine Berechnung sukzessiver Termen ermöglicht. Besteht die Ausgangsgeneration aus  $RR$ -Typen mit der Frequenz  $r^2 + \mathcal{A}_n$ ,  $RD$ -Individuen mit der Frequenz  $2rd - 2\mathcal{A}_n$  und  $DD$ -Individuen mit der Frequenz  $d^2 + \mathcal{A}_n$ , so erhält man die Frequenz von Kreuzungen und Nachkommen, die in Tabelle 11 angegeben wird.

Die Rekursionsformel erhält also folgendes Aussehen:

$$RR_n = r^2 + \frac{2kd^2 RR_{n-1}}{1 - RR_{n-1}} \quad (53)$$

$$RD_n = 2rd - \frac{4kd^2 RR_{n-1}}{1 - RR_{n-1}} \quad (54)$$

$$DD_n = d^2 + \frac{2kd^2 RR_{n-1}}{1 - RR_{n-1}} \quad (55)$$

Um den Grenzwert für die Serie zu erreichen, kann man folgendes Vorgehen anwenden. Man geht von Panmixie aus und berechnet die sukzessive Verschiebung während einer Anzahl von Generationen. Man geht ferner von einer Population aus, in welcher man sich vorstellt, dass eine totale Gattenwahl so lange stattgefunden hat, dass die Heterozygoten vollständig verschwunden sind. Berechnet man mit diesem Ausgangspunkt die Wirkung von partieller Gattenwahl mit Hilfe der oben angeführten Rekursionsformel, so erhält man eine sukzessive Zunahme der Heterozygotie und eine Abnahme der Homozygotie. Es zeigt sich nun, dass die Werte, welche man ausgehend von Panmixie erhält und falls man vom Grenzwert für die totale Gattenwahl mit einer bestimmten Anlagefrequenz ausgeht, bereits nach einer ziemlich geringen Anzahl von Generationen sehr nahe zueinander liegen. Zwischen den beiden Werten müssen die Grenzwerte des Prozesses liegen und nach 6—7 Generationen hat man diesen Wert innerhalb so enger Grenzen eingeschlossen, dass man in der Praxis keine genauere Vorstellung von dem Grenzwert zu haben braucht.

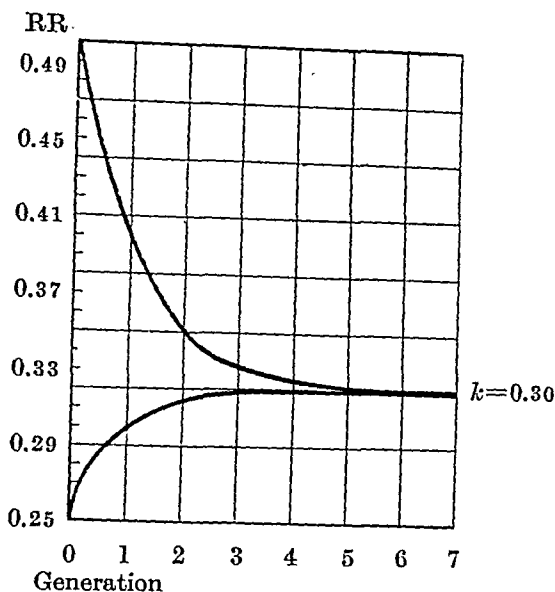


Abb. 18. Effekt von positiver partieller Gattenwahl in aufeinanderfolgenden Generationen, wenn die Intensität der Gattenwahl  $k = 0.30$  und die Frequenz der rezessiven Genes  $r = 0.5$  ist. (Vgl. Tabelle 12.) Die Berechnungen sind ausgeführt ausgehend von Panmixie und auch unter der Annahme, dass in der Population auf Grund von totaler Gattenwahl keine Heterozygoten existieren.

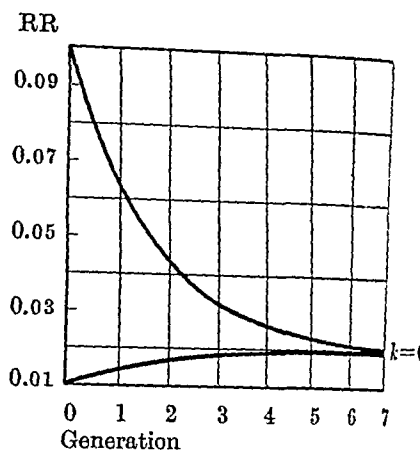


Abb. 19. Effekt von positiver partieller Gattenwahl in aufeinanderfolgenden Generationen, wenn die Intensität der Gattenwahl  $k = 0.30$  und die Frequenz des rezessiven Genes  $r = 0.1$  ist. (Vgl. Tabelle 12.) Die Berechnungen sind ausgeführt ausgehend von Panmixie und auch unter der Annahme, dass in der Population auf Grund von totaler Gattenwahl keine Heterozygoten existieren.

TABELLE 12.

Effekt verschiedener Intensität ( $k$ ) bei positiver, partieller Gattenwahl in aufeinanderfolgenden Generationen bei monohybrider, rezessiver Diallelie, wenn die rezessiven Eigenschaftsträger verschiedene Frequenz haben. Vgl. Formel 53.

Generation	$r^2 = 0.001$		$r^2 = 0.01$		$r^2 = 0.25$	
	$k = 0.10$	$k = 0.30$	$k = 0.10$	$k = 0.30$	$k = 0.10$	$k = 0.30$
0	0.00100	0.00100	0.0100	0.0100	0.2500	0.2500
1	0.00119	0.00156	0.0116	0.0149	0.2667	0.3000
2	0.00122	0.00188	0.0119	0.0174	0.2682	0.3143
3	0.00123	0.00206	0.0120	0.0186	0.2683	0.3188
4		0.00216		0.0192		0.3202
5		0.00222		0.0195		0.3207
6		0.00225		0.0197		0.3208
7		0.00227		0.0198		0.3209
8		0.00228				





TABELLE 13. Vgl. Text.

Eltern- kombina- tionen	Häufigkeit in der Population	Kinder		
		Häufigkeit der Typen		
		RR <sub>n</sub>	RD <sub>n</sub>	DD <sub>n</sub>
RR × RR <sub>n-1 n-1</sub>	$(r^2 + \Delta)h = a$	a		
RR × RD <sub>n-1 n-1</sub>	$\frac{2(r^2 + \Delta)(2rd - 2\Delta)(1-h)}{1 - (r^2 + \Delta)} = b$	$\frac{1}{2}b$	$\frac{1}{2}b$	
RR × DD <sub>n-1 n-1</sub>	$\frac{2(r^2 + \Delta)(d^2 + \Delta)(1-h)}{1 - (r^2 + \Delta)} = c$	c	c	
RD × RD <sub>n-1 n-1</sub>	$\frac{(2rd - 2\Delta)^2 \left[ 1 - \frac{(r^2 + \Delta)(1-h)}{1 - (r^2 + \Delta)} \right]}{\left[ 1 - \frac{(r^2 + \Delta)(1-h)}{1 - (r^2 + \Delta)} \right] [1 - (r^2 + \Delta)]} = d$	$\frac{1}{4}d$	$\frac{1}{2}d$	$\frac{1}{4}d$
RD × DD <sub>n-1 n-1</sub>	$\frac{2(2rd - 2\Delta)(d^2 + \Delta) \left[ 1 - \frac{(r^2 + \Delta)(1-h)}{1 - (r^2 + \Delta)} \right]}{1 - (r^2 + \Delta)} = e$		$\frac{1}{2}e$	$\frac{1}{2}e$
DD × DD <sub>n-1 n-1</sub>	$\frac{(d^2 + \Delta)^2 \left[ 1 - \frac{(r^2 + \Delta)(1-h)}{1 - (r^2 + \Delta)} \right]}{1 - (r^2 + \Delta)} = f$			f
Summen		RR <sub>n</sub> = $a + \frac{1}{2}b + \frac{1}{4}d$	RD <sub>n</sub> = $\frac{1}{2}b + c + \frac{1}{2}d + \frac{1}{2}e$	DD <sub>n</sub> = $\frac{1}{4}d + \frac{1}{2}e + f$

erhalten und dieselbe Formel, wie früher, gilt für diesen Prozess. (Ist  $h$  kleiner als  $r^2$ , so liegt eine negative Gattenwahl vor, vgl. weiter unten.) Es muss daran erinnert werden, dass  $h$  eine andere Bedeutung besitzt wie  $k$  in den früher angegebenen Formeln. Wir nehmen nun an, dass ein bestimmter konstanter Anteil der rezessiven Homozygoten miteinander durch Ehe vereint werden, — in den früheren Formeln wurde angenommen, dass ein konstanter Anteil von ehelichen Vereinigungen der Heterozygoten ausbleibt.

### Negative totale und partielle Gattenwahl.

Die Wirkung einer negativen Gattenwahl wurde früher nicht untersucht und, wie oben hervorgehoben, wurde nicht festgestellt, dass ein solcher Prozess in menschlichen Populationen vorkommt, wenn dies auch wohl vorstellbar ist. Es zeigt sich nun, dass für einen solchen Prozess dieselben Formeln gelten wie für die positive partielle Gattenwahl. In der Rekursionsformel ändern sich aber die Zeichen zwischen den Termen, wenn man annimmt, dass die Ehen zwischen den Heterozygoten um einen bestimmten Anteil  $h$  zunehmen. Die Formel wird also:

$$RR_{n+1} = r^2 - \frac{2kd^2 RR_n}{1 - RR_n} \quad (57)$$

Nimmt man hingegen an, dass ein bestimmter konstanter Anteil der rezessiven Homozygoten  $h$ , weniger als  $r^2$ , sich nicht miteinander verheiraten, so gilt die früher angegebene Rekursionsformel (56):

$$RR_{n+1} = RR_n h + \frac{2 RR_n (r - RR_n) (1 - h)}{1 - RR_n} + \frac{(r - RR_n)^2 \left[ 1 - \frac{RR_n (1 - h)}{1 - RR_n} \right]}{1 - RR_n}$$

Will man mit Hilfe dieser Formeln den Wert für die negative totale Gattenwahl erhalten, kann man in der letzteren Formel  $h = 0$  setzen. Hingegen kann man in der ersten Formel nicht  $k = 1$  setzen, da dies bedeutet, dass Ehen zwischen Personen mit entgegengesetzten Eigenschaften in gewissen Fällen die Frequenz der Eigenschaftsträger erhöhen würden, und zwar mit einem grösseren

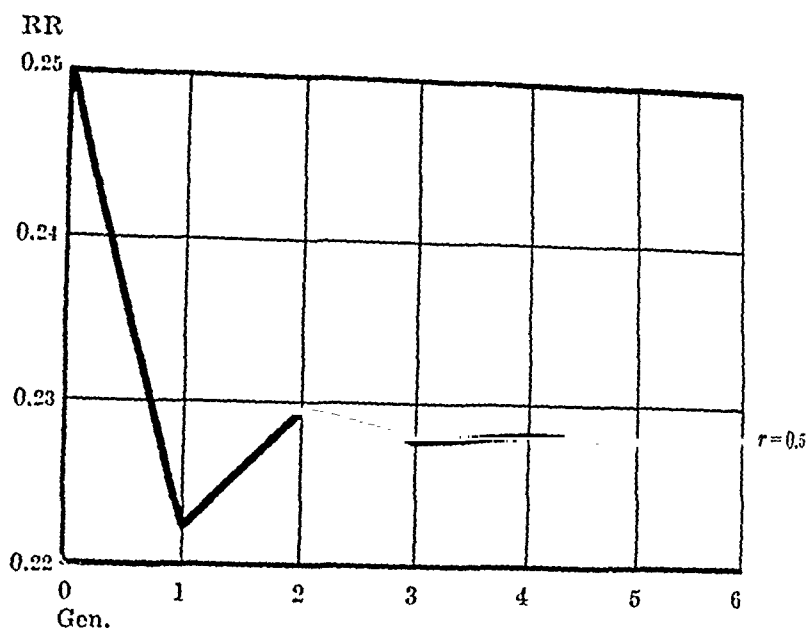


Abb. 20. Totale negative Gattenwahl in aufeinanderfolgenden Generationen, wenn der rezessive Gen die Frequenz  $r = 0.5$  hat. (Vgl. Tabelle 14.)

ren Anteil als unter Berücksichtigung der Frequenz der Eigenschaftsträger möglich ist. Bei negativer totaler Gattenwahl wird der Grenzwert

$$k = \frac{RR_{n-1}}{2(1 - RR_{n-1})} \quad (58)$$

Wird dieser Ausdruck in die erste Formel eingesetzt und setzt man in der zweiten Formel  $h = 0$ , so wird folgender Ausdruck für das

TABELLE 14.

Totale, negative Gattenwahl in aufeinanderfolgenden Generationen bei rezessiver, monohybrider Diallelie, wenn die Eigenschaftsträger verschiedene Frequenz haben. Vgl. Formel 59.

Generation	$r^2 = 0.001$	$r^2 = 0.01$	$r^2 = 0.1$	$r^2 = 0.25$
0	0.001000	0.01000	0.10000	0.2500
1	0.000999	0.00992	0.09423	0.2222
2		0.00999	0.09494	0.2296
3			0.09486	0.2278
4			0.09487	0.2282
5			0.09486	0.2281
6				0.2282

Verhältnis zwischen zwei aufeinander folgende Termen bei totaler negativer Gattenwahl erhalten:

$$RR_{n+1} = r^2 - \frac{d^2 RR_n^2}{(1 - RR_n)^2} \quad (59)$$

Mit Hilfe dieser Formeln wurden die Werte in Abb. 20 und Tabelle 14 berechnet. Es zeigt sich, dass die Heterozygoten während einer mässigen Anzahl Generationen zunehmen und dass die Serie gegen einen Grenzwert oszilliert. Dies beruht darauf, dass falls man bei Panmixie eine negative Gattenwahl einführt, die Heterozygoten in der ersten Generation wesentlich zunehmen. Dies bedingt, dass die nächste Generation in verhältnismässig grossem Ausmasse aus Ehen zwischen Heterozygoten stammt. Aus diesen Ehen stammen indessen verhältnismässig viele Homozygoten, weswegen ihre Frequenz in der dritten Generation grösser wird als in der zweiten Generation. Nach einer verhältnismässig geringen Anzahl von Generationen wird indessen eine Gleichgewichtslage erreicht, die aber nur in mässigem Grade von Panmixie abweicht. Ist z. B.  $r = \frac{1}{2}$  und kommen also die rezessiven Eigenschaftsträger in 25 % der Bevölkerung vor, so bedingt die totale negative Gattenwahl nach 3—4 Generationen eine Verschiebung zu einer stabilen Lage bei 22.8 %.

## **Gattenwahl, Verwandtschaftsehe, Selektion und Mutation.**

Positive Gattenwahl wirkt im Prinzip auf ähnliche Weise wie Verwandtschaftsehen, d. h. bedingt eine Zunahme der Homozygotie auf Kosten der Heterozygotie. Die Wirkung ist bei beiden Prozessen unter im übrigen gleichen Verhältnissen, stärker auf seltenere Eigenschaften als auf häufig vorkommende. Indessen muss daran erinnert werden, dass man sich nicht vorstellen kann, dass die Gattenwahl für sehr seltene Eigenschaften eine grössere Rolle spielen kann, weil ja in diesem Falle die Eigenschaftsträger keine Aussicht haben in nennenswertem Ausmasse einander zu finden. Es ist deswegen kaum wahrscheinlich, dass Gattenwahl für menschliche Populationen eine grössere Rolle spielt und die Abweichung von Panmixie, welche Verwandtschaftsehen zusammen mit Gattenwahl bewirken, dürfte in den meisten Fällen als bedeutungslos angesehen werden. Es ist deswegen auch nicht wahrscheinlich, dass diese Prozesse wesentlicher die Heterozygotenfrequenz bei der Gleichgewichtslage beeinflussen werden, die in einer gegebenen Situation zwischen Mutationen und Selektion vorliegt. Es ist aber nicht möglich, gegenwärtig zu einem definitiven zahlenmässigen Mass für die Bedeutung dieser verschiedenen Prozesse zu gelangen, wenn sie gleichzeitig stattfinden, da eine empirische Unterlage für die Berechnungen fehlt. Wir werden das Problem doch in einem folgenden Abschnitt etwas eingehender besprechen.

## **Die Bedeutung der Isolate für die Zusammensetzung von Populationen.**

Im Vorhergehenden haben wir bei der Aufstellung von Formeln als Ausgangspunkt eine Population mit Panmixie angewandt. Wir haben aber den Begriff Population nicht näher diskutiert. Streng genommen ist in diesem Zusammenhange eine Population die Volks-

menge, innerhalb welcher Panmixie herrscht. In der Praxis können wir selbstverständlich mit Sicherheit feststellen, dass eine Panmixie so gut wie niemals über die politischen Grenzen eines Landes hinaus vorkommt. Die Annahme scheint mehr angebracht zu sein, dass innerhalb einer Nation Panmixie vorhanden ist. Es ist aber klar, dass, besonders wenn es sich um eine grössere Nation handelt, diese Annahme nicht berechtigt ist. Eine Person, die in einem bestimmten Teil des Landes wohnt, hat die Möglichkeit sich innerhalb eines bestimmten begrenzten Gebietes des Landes und innerhalb einer bestimmten sozialen Schicht zu verheiraten. Eine Nation, eine grosse Population, besteht also aus Teilpopulationen, kleineren Einheiten, innerhalb welcher man ein grösseres Recht besitzt anzunehmen, dass eine Panmixie vorhanden ist. Die Teilpopulationen, aus welchen also eine grössere Nation besteht, werden Isolate genannt. Die Grenzen, welche hierbei in Frage kommen, sind teils geographischer Art, z. B. Wälder, Flüsse, teils sozialer Art. Eine Person, die einer bestimmten sozialen Schicht angehört, hat eine geringere Möglichkeit sich in einer Schicht oberhalb oder unterhalb der eigenen zu verheiraten.

### Grösse der Isolate.

Es ist nun von einem gewissen Interesse eine Vorstellung über die Grösse der Isolate zu erhalten. Dies kann man, wie von DAHLBERG (1929) gezeigt wurde, mit Hilfe der Frequenz an Verwandtschaftsehen erreichen. Wir haben vorher hervorgehoben, dass bei Panmixie Verwandtschaftsehen zufallsbedingt auftreten müssen. Die Frequenz an Verwandtschaftsehen wird natürlich teilweise von der Grösse der Bevölkerung und teilweise von der Grösse der Familien abhängen. Wir nehmen an, dass die durchschnittliche Anzahl an Kindern in jeder Familie, die das erwachsene Alter erreicht und eine Ehe schliesst,  $b$  ist, sowie dass die Anzahl Individuen dieser Art in der ganzen Bevölkerung  $n$  ist.

Wählt man nun eine Person rein zufällig aus der Bevölkerung aus, so besitzt sie im Durchschnitt  $b(b-1)$  Vettern, bzw. Basen, mit denen sie sich verheiraten kann. Die gesamte Anzahl an Individuen, mit denen sie sich verheiraten kann, ist  $\frac{n-1}{2}$ . Die Wahrscheinlichkeit für eine Vetternehe  $c$  ist also:

$$c = \frac{2b(b-1)}{n-1} \text{ und } n-1 = \frac{2b(b-1)}{c} \quad (60)$$

Bei der Aufstellung dieser Formel mussten wir den Geschlechtsunterschied nicht berücksichtigen. Eine Person kann sich ja mit einer anderen Person des gleichen Geschlechtes nicht verheiraten. Dieser Faktor wirkt in gleich starkem Grade auf das Eingehen einer Ehe hemmend, wenn es sich um Verwandte, wie um andere Personen in der Bevölkerung handelt. Aus diesem Grunde muss man den Geschlechtsunterschied nicht berücksichtigen. Im Hinblick auf den Altersunterschied zwischen den Individuen einer Bevölkerung kann man vielleicht wagen zu behaupten, dass dieser eher für die gesamte Anzahl von Individuen in einer Bevölkerung als für Vettern und Basen Bedeutung besitzt. Wählt man mit anderen Worten wahllos zwei Vettern aus, so ist deren wahrscheinlicher Altersunterschied geringer als der Altersunterschied, den zwei wahllos ausgewählte, nicht miteinander verwandte, unverheiratete Personen aufweisen. Dies bedingt, dass der faktische Frequenzwert für Vetternehen etwas grösser sein muss, als es die Formel erfordert. Schliesslich soll hervorgehoben werden, dass die Vettern bzw. Basen einer bestimmten Person auf Grund von Ortswechsel, der in einer Bevölkerung vorkommt, ausserhalb der Grenzen des Isolates leben können, zu welchem die betreffenden Personen gehören. Dieser Faktor wirkt also auf die Weise, dass ein bestimmter Vetter oder eine bestimmte Base eine etwas geringere Möglichkeit hat sich mit einer seiner Basen oder einem seiner Vettern zu verheiraten, als dies die Formel fordert; dies wirkt also gegen die Faktoren, die wir vorher besprochen haben. Es ist wohl gegenwärtig unmöglich, für die Bedeutung dieser Faktoren ein exaktes Mass zu erhalten. Es ist aber *a priori* kaum wahrscheinlich, dass die betreffenden Faktoren eine allzu grosse Bedeutung haben würden und wir können wohl annehmen, dass der Frequenzwert für die Vetternehen, den wir empirisch finden, wenigstens bei einigen Populationen einigermaßen mit dem übereinstimmt, was man gemäss der Formel zu erwarten hat. Die Kinderanzahl in Westeuropa ist ungefähr 2 pro Ehe. Es soll daran erinnert werden, dass es sich um erwachsene Kinder handelt, welche eine Ehe schliessen. Ist diese Anzahl im Durchschnitt zwei pro Familie, so hält sich die Bevölkerung konstant. Ist die Anzahl drei, so nimmt die Bevölkerung in einer Generation um 50 % zu. Nachdem die Populationen

TABELLE 15.

Isolatgrösse ( $=n$ ) bei zufallsbedingtem Vorkommen von Vetternehen in verschiedener Häufigkeit ( $=c$ ). Vgl. Formel 60.

$c$	$n$
0.0001	40 000
0.001	4 000
0.0025	1 600
0.005	800
0.01	400
0.05	80
0.1	40

in ihrer Grösse nahezu konstant sind, bedeutet dies, dass die Kinderanzahl ungefähr zwei ist. Nimmt man die Kinderanzahl zu zwei, so findet man bei verschiedener Frequenz an Vetternehen die Werte, welche in Tabelle 15 angegeben sind. Es soll aber daran erinnert werden, dass diese Werte approximativ sind. Wir haben angenommen, dass die Familiengrösse konstant ist oder unbedeutend variiert. Variiert die Grösse beträchtlicher, so muss dies bedeuten, dass man eine grössere Frequenz an Vetternehen zu erwarten hat, was seinerseits bedeutet, dass die Werte, die in der Tabelle angegeben werden, durchgehend etwas zu niedrig sind. Es würde indessen kaum von Interesse sein korrektere Berechnungen auszuführen, da es sich ja nur darum handelt, eine Vorstellung über die Grössenordnung der Isolate zu erhalten. In diesem Zusammenhang muss hervorgehoben werden, dass der Isolatbegriff, um den es sich hier handelt, eine Schematisierung der Situation darstellt. Streng genommen fallen die Isolate für zwei Personen niemals vollständig zusammen und das Isolat für gewisse Personen geht allmählich in der Population auf, ohne dass man immer von scharfen Grenzen sprechen kann. Eine andere Person des entgegengesetzten Geschlechtes hat geringere Aussichten mit ihr die Ehe zu schliessen, je grösser der Abstand zwischen beiden ist. Geht der Abstand über eine gewisse Grenze hinaus, so ist praktisch genommen keine Möglichkeit für eine Ehe zwischen den betreffenden Personen vorhanden. Eine Gruppe von Personen, die nahe zueinander wohnen, hat indessen im grossen und ganzen ein gemeinsames Isolat. Zwischen den Isolaten der verschiedenen Personen sind mit anderen Worten die Unterschiede unbedeutend. Die Verhältnisse sind selbstverständlich in den verschie-



denen Populationen unterschiedlich. In einigen finden sich scharfe Isolatgrenzen, in anderen sind die Grenzen weniger scharf, aber auch in den letzteren ist selbstverständlich insofern eine Isolierung vorhanden, dass eine Person Aussicht hat, eheliche Verbindungen nur mit einer begrenzten Anzahl von Personen einzugehen. Es muss ferner daran erinnert werden, dass ein Isolat zeitlich nicht scharf begrenzt ist. Die Generationen gehen ohne scharfe Grenzen ineinander über. Auch hierbei haben wir uns erlaubt zu schematisieren. Die Isolate ebenso die Generationen, mit denen wir rechnen, sind also fiktiv und sind fest abgegrenzte Einheiten, welche die unscharfen Isolate und Generationen ersetzen, die in Wirklichkeit vorhanden sind. Es ist notwendig sich hieran zu erinnern, wenn man den Isolatbegriff anwendet.

Wenn es sich darum handelt, eine Vorstellung über die Isolatgrösse zu erhalten, so muss vor allem daran erinnert werden, dass diese selbstverständlich in hohem Grade variiert. Das eine Extrem stellt die Grosstadt mit ihren beweglichen Menschenmassen dar, das andere ein Land mit Einödecharakter, z. B. im nördlichen Europa, mit kleinen isolierten Dörfern. Hierzu kommt, dass die Grösse der Isolate gegenwärtig auf Grund der Entwicklung der Kommunikationen, der Flucht in die Städte u. s. w. grossen Verschiebungen unterworfen ist. Mit Hilfe der zugänglichen Werte dürfte man davon ausgehen können, dass Vetternehen in älterer Zeit nahezu in 1 % vorkamen, was einer Isolatgrösse von 400 Individuen entspricht. Gegenwärtig scheinen sich Vetternehen in ihrer Frequenz 0.25 % zu nähern, was einer Isolatgrösse von 1600 Individuen entspricht. Letzteres würde bedeuten, dass eine Person im Durchschnitt jetzt die Möglichkeit hat bei der Eheschliessung unter 800 Personen anstatt früher unter nur 200 Personen zu wählen.

Es ist ja klar, dass wenn zwei Isolate, zwischen welchen ein Austausch stattfindet, verschiedene Beschaffenheit aufweisen, dieser Austausch einen Ausgleich des Unterschiedes zwischen den Isolaten bewirken muss, falls bei den Kreuzungen, die hinaus über die Isolatgrenzen stattfinden, keine Selektion stattfindet. Es ist klar, dass falls es sich um soziale Isolatgrenzen handelt, man mehr Anlass hat, eine solche Selektion zu erwarten, als wenn es sich um geographische Isolatgrenzen handelt. Liegt aber keine Selektion vor, so müssen, wie bereits hervorgehoben, Kreuzungen über die Isolatgrenzen hinaus bedingen, dass der evt. vorhandene Unterschied zwischen den Iso-

laten im Hinblick auf die Vererbung ausgeglichen wird. Nachdem dies geschehen ist, besitzen die Isolatgrenzen keine Bedeutung mehr. Wir können dann damit rechnen, dass für die gesamte Population eine Panmixie vorliegt.

Es ist nun am interessantesten zu untersuchen, welches Ergebnis erhalten wird, wenn die Isolate eine voneinander abweichende Beschaffenheit haben.

### Die Bedeutung der Isolatgrenzen bei monohybrider Diallelie.

Wir gehen von einer Population aus, die aus zwei Isolaten besteht, zwischen welchen keine Kreuzungen stattfinden. In jedem der Isolate ist Panmixie vorhanden. Wir nennen die Isolate  $G$  und  $H$  und nehmen an, das sich deren Grösse wie  $g : h$  verhält und dass  $g + h = 1$  ist. Die Gameten mit der rezessiven Anlage  $R$  haben in  $G$  die Frequenz  $r_g$  und in  $H$  die Frequenz  $r_h$ . Die Frequenzen der entsprechenden dominanten Anlage  $D$  sind  $d_g$  bzw.  $d_h$ , wobei  $r_g + d_g = 1$  und  $r_h + d_h = 1$ . Die Zusammensetzung der Isolate ergibt sich aus Tabelle 16 (welche ebenso wie Tabelle 17 aus einer Arbeit von WAHLUND 1928 stammt. WAHLUND ist der erste, der diese Probleme behandelt hat. Wir folgen hier seiner Darstellung).

TABELLE 16.

Vgl. Text.

Zygotenarten	Proportionen	
	in $G$	in $H$
Rezessive Homozygoten, $RR$ .....	$r_g^2$	$r_h^2$
Heterozygoten, $RD$ .....	$2 r_g d_g$	$2 r_h d_h$
Dominante Homozygoten, $DD$ .....	$d_g^2$	$d_h^2$
Rezessive Merkmalsträger, $RR$ .....	$r_g^2$	$r_h^2$
Dominante Merkmalsträger, $RD+DD$ ..	$1 - r_g^2$	$1 - r_h^2$

Die Verhältnisse in der gesamten Population erhält man dadurch, dass man die Isolate proportional zu ihrer Grösse zusammenschlägt. Das Ergebnis ist aus Tabelle 17 ersichtlich.

TABELLE 17.

Vgl. Text.

Zygotenarten	Proportionen der Gesamtpopulation ( $G + H$ )		
	Tatsächlich (1)	Aus ihren Gametenproportionen berechnet (2)	Überschuss (1) — (2) = (3)
Rezessive Homozygoten, RR .....	$gr_g^2 + hr_h^2$	$(gr_g + hr_h)^2$	$+ gh (r_g - r_h)^2$
Heterozygoten, RD ...	$2 gr_g d_g + 2 \cdot hr_h d_h$	$2 (gr_g + hr_h) \cdot (gd_g + hd_h)$	$- 2 gh (r_g - r_h)^2$
Dominante Homozygoten, DD .....	$gd_g^2 + hd_h^2$	$(gd_g + hd_h)^2$	$+ gh (r_g - r_h)^2$
Rezessive Merkmals-träger, RR .....	$gr_g^2 + hr_h^2$	$(gr_g + hr_h)^2$	$+ gh (r_g - r_h)^2$
Dominante Merkmals-träger RD+DD ....	$1 - gr_g^2 - hr_h^2$	$1 - (gr_g + hr_h)^2$	$- gh (r_g - r_h)^2$

Wir erhalten also in der gesamten Population mehr Homozygoten ( $RR$  bzw.  $DD$  Individuen), als wir erhalten haben würden, falls in der ganzen Population eine Panmixie vorgelegen wäre und keine Isolatgrenzen vorhanden gewesen wären (Kolumne 2). Der Überschuss an jeder Homozygotenart wird durch den immer positiven Ausdruck  $gh(r_g - r_h)^2$  bestimmt. Dieser Ausdruck gibt auch die Zunahme an rezessiven Eigenschaftsträgern und gibt verdoppelt die Abnahme der Heterozygoten an.

Abb. 21 gibt graphisch eine Darstellung des Homozygotenüberschusses unter verschiedenen Verhältnissen. Entlang der Abszisse werden die Unterschiede zwischen den Gametenproportionen ( $r_g - r_h$ ) in den Isolaten abgelesen. Die Ordinaten der Kurve geben die Homozygotenzunahme gemäss des Ausdrucks an, den wir oben angeführt haben.

Je grösser die Unterschiede zwischen den Gametenproportionen in den beiden Isolaten sind, um so grösser wird die Homozygotenzunahme. Je kleiner der Grössenunterschied zwischen den Isolaten ist, desto grösser wird auch der Überschuss. Die maximale Wirkung der Isolate wird erhalten, wenn die Isolate gleich gross sind, sowie wenn der Unterschied zwischen den Gametenproportionen in den Isolaten 1 ist. In diesem Falle wird jedes der Isolate aus der entsprechenden Homozygotenart bestehen. Heterozygoten sind dann nicht vorhanden. Sind die Isolate gleich gross, müssten bei dieser

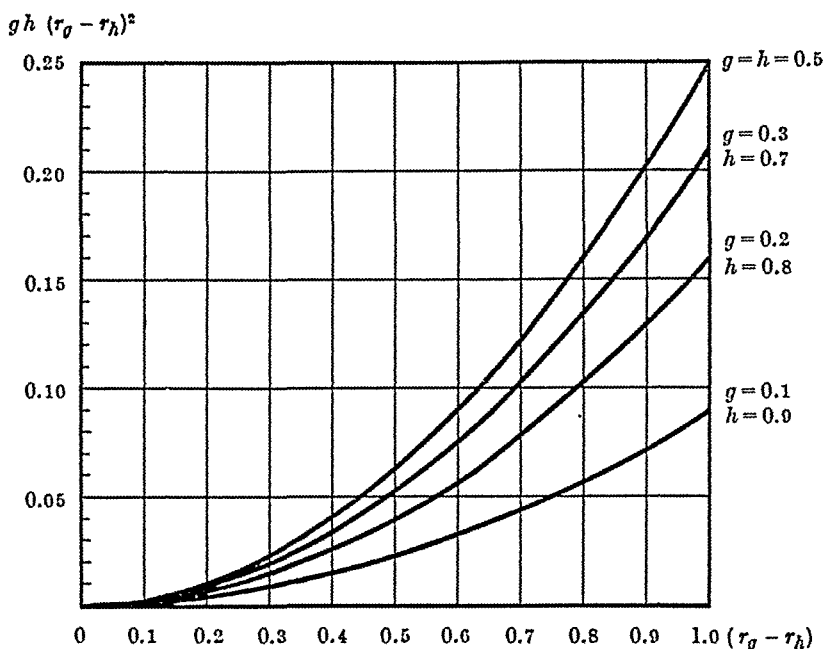


Abb. 21. Unterschied  $g h (r_g - r_h)^2$  zwischen den tatsächlichen und den aus den Gametenproportionen berechneten Homozygotenproportionen  $RR$  oder  $DD$ , wobei der Unterschied  $(r_g - r_h)$  zwischen den Gametenhäufigkeiten zweier in der Population enthaltener Isolate und den Anteilen der beiden Isolate an der Totalpopulation  $g$  und  $h$  variiert.

Gametenbeschaffenheit 50 % Heterozygoten erhalten werden und der Wegfall derselben ist also die maximale Wirkung, welche die Isolate haben können. Je kleiner das eine Isolat im Verhältnis zum anderen ist und je geringer der Unterschied in ihren Genzusammensetzungen ist, desto geringer ist die Bedeutung der Isolatgrenzen.

Man kann auf analoge Weise zeigen, dass falls man  $m$  Isolate hat, welche proportional die Größen  $i_1 i_2 i_3 \dots i_m$  mit dem Gehalt an Genen  $r_1 r_2 r_3 \dots r_m$  sowie eine Frequenz  $r$  der  $R$ -Gene in der ganzen Population haben, so wird der Überschuss an rezessiven (oder dominanten) Homozygoten:

$$i_1 (r - r_1)^2 + i_2 (r - r_2)^2 + i_3 (r - r_3)^2 + \dots i_m (r - r_m)^2 = \sigma_i^2 \quad (61)$$

Diesen Ausdruck nennen wir  $\sigma_i^2$ . Er stimmt nämlich mit dem Quadrat der Standardabweichung für eine Serie, zusammengesetzt aus den Gametenproportionen in den verschiedenen Isolaten, proportional zur Grösse der Isolate, überein.

Diese Tatsache besitzt nun praktisches Interesse. Nimmt man eine empirische Untersuchung einer bestimmten Bevölkerung vor und teilt das untersuchte Material in mehrere Distrikte ein, so muss man eine gewisse Variabilität im Gehalt der rezessiven monohybriden Eigenschaftsträger finden, die auf einer zufallsbedingten Variation beruht, deren Grösse durch die Grösse des Materials bedingt wird. Die Standardabweichung, die aus dieser Quelle stammt, nennen wir  $\sigma_n$ . Wir erhalten ferner eine Variabilität, die durch einen evt. unterschiedlichen Gengehalt in den verschiedenen Isolaten bedingt wird. Für diese Standardabweichung haben wir oben einen Ausdruck gefunden und wir haben sie mit  $\sigma_i$  bezeichnet. Die zusammengesetzte empirisch gefundene Standardabweichung  $\sigma_e$  ist also aus zwei Arten von Standardabweichung zusammengesetzt, und zwar gemäss der Formel:

$$\sigma_e = \sqrt{\sigma_n^2 + \sigma_i^2} \quad (62)$$

Die Standardabweichung, die man auf Grund der Grösse des Materials und des durchschnittlichen Gengehalts zu erwarten hat, kann ja leicht theoretisch berechnet werden. Da man also  $\sigma_n$  und  $\sigma_e$  in der obenstehenden Gleichung kennt, kann man die Abweichung, welche die Isolate bedingen, berechnen und erhält ein Mass für sie.

Hat man nun ein grosses Material zur Verfügung, so wird die Standardabweichung, welche durch die begrenzte Grösse des Materials bedingt wird, verschwindend klein. Die empirisch gefundene Standardabweichung wird dann so gut wie ausschliesslich durch diejenige Standardabweichung konstituiert, welche durch die Verschiedenheit der Isolate in der Bevölkerung bedingt wird. Hat man ein sehr grosses Material, kann die Standardabweichung, die man bei der Aufteilung in kleinere, aber nicht zu kleine Distrikte erhält, so betrachtet werden, dass sie so gut wie ausschliesslich auf dem Homozygotenüberschuss beruht, der durch die Isolatgrenzen bedingt wird und für ihn einen Ausdruck darstellt. Ist also diese Standardabweichung klein oder 0, so besitzen die Isolatgrenzen keine Bedeutung, die Bevölkerung ist im Hinblick auf die Vererbung homogen.

In einer solchen Bevölkerung haben die rezessiven Homozygoten die Frequenz  $r^2$ , die dominanten Homozygoten eine solche von  $d^2$  und die Frequenz der Heterozygoten beträgt  $2rd$ . Aus der Frequenz der rezessiven Eigenschaftsträger kann man also  $r$  berechnen. Haben die Isolatgrenzen Bedeutung und haben die Isolate also einen von-

einander abweichenden Gehalt an Genen, so erhält man durch eine solche Berechnung einen falschen Wert für die Frequenz der *R*-Gameten. Der richtige Wert für diese Frequenz  $r_i$  wird erhalten, wenn die Frequenz der rezessiven Eigenschaftsträger  $r^2$  genannt wird und man  $\sigma_i^2$  auf die oben angegebene Art aus dem Ausdruck

$$r_i = \sqrt{r^2 + \sigma_i^2} \quad (63)$$

berechnet. Der korrigierte Wert für die Frequenz der *D*-Gene wird auf folgende Weise erhalten:

$$1 - \sqrt{r^2 + \sigma_i^2} \quad (64)$$

Im grossen und ganzen dürfte man erwarten, dass die Isolatgrenzen vor allem Bedeutung haben würden, wenn es sich um seltene Eigenschaften handelt. Handelt es sich um häufig vorkommende Eigenschaften (falls man Rassenunterschiede ausnimmt, vgl. weiter unten), so ist es nicht so wahrscheinlich, dass Unterschiede zwischen den Isolaten in dem Ausmasse vorliegen, dass sie wesentliche Abweichungen von dem verursachen, was man bei Panmixie über die gesamte Bevölkerung zu erwarten hat. Gerade das, dass eine Eigenschaft häufig vorkommt, d. h. allgemein vorkommt, schliesst ja in gewissem Masse grössere Unterschiede zwischen den Isolaten in einer Population aus. Ist hingegen eine Eigenschaft selten, muss sie ja gerade auf Grund ihrer Seltenheit nur in einigen Isolaten vorkommen können, während sie in anderen fehlt. In einer solchen Situation muss man die Isolatgrenzen berücksichtigen, wenn man die Frequenz der Heterozygoten berechnen will. Untersucht man z. B., wie sich das Vorkommen einer seltenen Krankheit in einem Land gestaltet, und findet man, dass sie nur in begrenzten Herden auftritt, so darf man selbstverständlich bei der Berechnung der Heterozygotenfrequenz nicht eine Panmixie über die gesamte Bevölkerung voraussetzen. Man erhält dann nämlich evident einen viel zu hohen Wert für das Vorkommen der Heterozygoten. Man muss die Berechnung auf die Isolate begrenzen, in welchen die Krankheit vorkommt und tut man dies, so erhält man eine wesentlich niedrigere Heterozygotenfrequenz für das gesamte Land, als man sie nach der fehlerhaften Berechnung erhält. (SjÖGREN hat z. B. 1931 die Heterozygotenfrequenz für juvenile amaurotische Idiotie in Schweden zu 1 % berechnet. Der richtige Wert ist ein Bruchteil davon.)

Die Isolatgrenzen besitzen u. a. deswegen Interesse, weil sie erklären, warum man schwere, seltene, rezessiv erbliche Krankheiten in abgelegenen und abgegrenzten Dörfern vorfindet. Um dies zu verstehen, muss man sich nur Folgendes überlegen. Entsteht eine Mutation in der Bevölkerung eines bestimmten Isolates und breitet sie sich hauptsächlich nur dort aus, (aber kaum ausserhalb des Isolates), so ist die Wahrscheinlichkeit dafür, dass die Anlage in doppelter Dosis zusammentreffen wird, grösser, wenn das Isolat klein ist, als wenn das Isolat gross ist. In einem kleinen, abgelegenen Dorf mit schlechten Kommunikationen sind die Verhältnisse für das Auftreten von Krankheiten dieser Art günstig. In grossen Isolaten, z. B. in einer Grosstadt, ist hingegen die Aussicht, dass eine solche Anlage sich manifestieren wird, klein. Aus diesem Grunde findet man schwere Nervenkrankheiten, Idiotieformen und ähnliches in Dörfern, die weit abgelegen sind und schlechte Kommunikationen besitzen. In diesen Dörfern ist die Lebensweise in der Regel ziemlich primitiv. Die Bevölkerung macht den Eindruck auf einem niedrigen kulturellen Niveau zu stehen. Man knüpft in diesem Falle gerne die erbliche Krankheit mit dem primitiven kulturellen Standpunkt zusammen und deutet mehr oder minder bestimmt an, dass es sich um eine Degeneration handelt, die beide Erscheinungen verursacht und ihre Ursache in Verwandtschaftsehen hat. In Wirklichkeit ist der Mangel an Kommunikationen die Ursache, da durch sie die Isolatgrenzen erhalten bleiben. Das kulturelle Niveau hat selbstverständlich aus Vererbungsgesichtspunkten keine Bedeutung. Man kann natürlich sagen, dass Verwandtschaftsehen eine Rolle spielen, es handelt sich aber um Verwandtschaftsehen, deren Frequenz nur dem zu entsprechen braucht, was man bei Panmixie innerhalb des Isolates zu erwarten hat.

### Aufhebung der Isolate bei Monohybridität.

Diese Gesichtspunkte führen auf die Frage über, welche Bedeutung einer Aufhebung der Isolatgrenzen möglicherweise bedingen kann. Wir untersuchen erst, welche Bedeutung die Aufhebung der Isolate bei monohybrider Diallelie besitzt, und zwar unter der Voraussetzung, dass es sich um eine seltene Anlage handelt, die in den umliegenden Isolaten fehlt. Vgl. DAHLBERG 1938. Ist die Frequenz des Gens  $r$ ,

und vergrößert sich das Isolat um  $a$  Teile von sich selbst, so wird die Genfrequenz in dem neuen Isolat  $\frac{r}{1+a}$  und die Frequenz der Eigenschaftsträger

$$RR = \frac{r^2}{(1+a)^2}$$

Wird mit anderen Worten die Isolatgröße verdoppelt, so wird die Frequenz der Eigenschaftsträger auf  $\frac{1}{4}$  der ursprünglichen Frequenz sinken. Nimmt aber die Frequenz der Eigenschaftsträger auf diese Weise ab, so muss man sich vom Standpunkt der Population aus daran erinnern, dass die Isolatgröße auf  $1+a$  zugenommen hat. Die niedrigere Frequenz wirkt sich also auf das vergrößerte Isolat so aus, dass vom Standpunkt der Population aus die Frequenz der Eigenschaftsträger nach der Formel

$$RR = \frac{r^2}{1+a} \quad (65)$$

abgenommen hat. Dies bedeutet, dass die Frequenz der Eigenschaftsträger in einer Population proportional zur Aufhebung der Isolate innerhalb der Population abnimmt. Werden die Isolate an Größe verdoppelt, so nimmt die Frequenz der Eigenschaftsträger auf die Hälfte ab. Im Hinblick auf die Frequenz monohybrider, rezessiver Defekte geht mit anderen Worten gegenwärtig ein Prozess von sehr entscheidender Bedeutung vor sich. Auf Grund der Industrialisierung, der Entwicklung der Kommunikationen und des Zuzuges in die Städte, werden die Isolate aufgehoben, was u. a. daraus ersichtlich ist, dass die Frequenz an Vetternehen abnimmt, was bereits früher hervorgehoben wurde. Dass Vetternehen seltener werden, wird u. a. teilweise durch die Vergrößerung der Bevölkerung bedingt. Nimmt ein Volk an Zahl zu, so müssen ja auch die einzelnen Isolate an Größe zunehmen. Die Zunahme, welche durch die Vergrößerung der Isolate innerhalb ihrer eigenen Grenzen bedingt wird, ist für die Frequenz der Anlagen innerhalb der Isolate ohne Bedeutung, falls nicht Selektion oder Mutationen hierbei Verschiebungen verursachen. Auch wenn man damit rechnet, dass ein Teil der verminderten Frequenz an Vetternehen durch eine Zunahme der Bevölkerung bedingt wird, so muss die Verminderung doch zum wesentlichen Teil auf einer Aufhebung der Isolate beruhen. Will



man vorsichtig sein, so kann man damit rechnen, dass die Isolate wenigstens an Grösse verdoppelt werden und vielleicht dreifache Grösse angenommen haben, beurteilt nach den Werten für die Frequenz an Vetternehen in gewissen Teilen Deutschlands. Dies muss mit sich gebracht haben, dass die Frequenz seltener, monohybrider, rezessiver Defekte auf etwa die Hälfte oder ein Drittel gesunken ist. (Dass in der offiziellen Statistik die Frequenz defekter Individuen zunimmt, dürfte mit einer besseren Registrierung zusammenhängen. Teilweise sind sie ja übrigens milieubedingt.)

Schliesslich soll hervorgehoben werden, dass der Unterschied zwischen Stadt und Land im Hinblick auf die Frequenz des Vorkommens defekter Individuen teilweise dadurch bedingt wird, dass die, welche in die Städte einwandern, ein ausgewähltes Material darstellen, in welchem die Defekten unterrepräsentiert sind. Zum Teil beruht der Unterschied zwischen Stadt und Land sicher darauf dass die Städte grössere Isolate sind, in welchen die erblich bedingten Anlagen zu Heterozygoten verdünnt werden und also eine geringere Aussicht für ein Zusammentreffen im homozygoter Form haben.

Wir haben die Aufhebung der Isolate unter der Annahme untersucht, dass es sich um sehr seltene Anlagen handelt, die nur in einer kleineren Anzahl von Isolaten in einer Bevölkerung vorkommen, gleichzeitig, wie wir angenommen haben, dass die Isolate auf Grund der Isolataufhebung an Grösse zunehmen. Man kann einen Schritt weiter gehen und die Wirkung einer Isolataufhebung untersuchen, wenn nur Unterschiede in der Anlagefrequenz zwischen den verschiedenen Isolaten vorliegen. (Vgl. WAHLUND 1928.) Der Einfachheit halber nehmen wir an, dass ein Austausch zwischen den Isolaten stattfindet, ohne dass der Anteil an der Bevölkerung verändert wird, den sie ausmachen. Wir nehmen also an, dass in einer konstanten Bevölkerung die Isolate konstant sind, was bedeutet, dass einer Einwanderung in ein bestimmtes Isolat eine Auswanderung von derselben Grössenordnung entspricht. Nehmen wir an, dass in einem bestimmten Isolat die Anlage die Frequenz  $r_s$  besitzt und dass ein bestimmter Teil des Isolates  $a$  aus Einwanderern besteht, die gegen die Auswanderer ausgetauscht wurden. Die Eingewanderten haben den Anlagegehalt  $r_{x_1}$ . Man erhält dann in der ersten Generation folgende Proportion der Anlage:

$$r_{s_1} = (1 - a_s) r_s + a_s r_{x_1}.$$

In der nächsten Generation erhält man die Proportion:

$$r_{s_2} = (1 - a_{s_1}) r_{s_1} + a_{s_1} r_{x_1}$$

und nach  $n$  Generationen erhält man:

$$r_{s_n} = (1 - a_{s_n}) r_{s_{(n-1)}} + a_{s_n} r_{x_n}. \quad (66)$$

Ist der Anlagegehalt  $r_x$  der Einwanderer konstant, so wird nach  $n$  Generationen der Unterschied zwischen dem Anlagegehalt des Isolates und dem der Einwanderer der folgende:

$$r_{s_n} - r_x = (1 - a_{s_n}) (r_{s_{(n-1)}} - r_x).$$

Die gesamte Verschiebung wird dann:

$$r_{s_n} - r_x = (1 - a_{s_1}) (1 - a_{s_2}) (1 - a_{s_3}) \cdots (1 - a_{s_n}) (r_s - r_x);$$

und setzt man

$$1 - a_s = \sqrt[n]{(1 - a_{s_1}) (1 - a_{s_2}) (1 - a_{s_3}) \cdots (1 - a_{s_n})},$$

so erhält man folgende Formel:

$$r_{s_n} - r_x = (1 - a_s)^n (r_s - r_x). \quad (67)$$

Wenn  $a_s$  ein Bruch ist, nähert sich  $(1 - a_s)^n$  bei zunehmendem  $n$  immer mehr dem Wert 0, so dass allmählich der Unterschied im Anlagegehalt zwischen den Einwanderern und dem Isolat verschwindet und die Verschiebung geht erst rascher, später langsamer.

Man kann nun eine Einwanderung von verschiedener Grösse und mit einem variierenden Gehalt der Anlage aus einer Bevölkerung in einem Isolat mit einer Einwanderung von variierender Grösse, aber konstantem Gehalt, entsprechend dem, den die Bevölkerung aufweist, ersetzen. Hierbei muss man im Hinblick auf den Anteil, den das Isolat in einer Bevölkerung ausmacht, korrigieren. In einem bestimmten Isolate, zum Beispiel in dem  $s$ -ten, dessen Anteil an der Gesamtpopulation als  $i_s$  angenommen ist und dessen ursprüngliche rezessive Gametproportion mit  $r_s$  bezeichnet wird, wird die erste Einwanderung, wie leicht gezeigt werden kann, folgenden Charakter haben

$$\frac{1}{1 - i_s} r - \frac{i_s}{1 - i_s} r_s$$

Die Proportion der zweiten Einwanderung

$$\frac{1}{1-i_s} r - \frac{i_s}{1-i_s} r_{s_1}$$

u. s. w. Es wird ja nämlich vorausgesetzt, dass ein Ausgleichsprozess vor sich geht und weiter, dass in jedem Isolate die Einwanderung eine representative Auswahl aus den übrigen darstellt. Daher kann man ersetzen:

$$a_{s_1}, a_{s_2}, a_{s_3}, \dots a_{s_n}$$

mit

$$\frac{a_{s_1}}{1-i_s}, \frac{a_{s_2}}{1-i_s}, \frac{a_{s_3}}{1-i_s} \text{ bzw. } \frac{a_{s_n}}{1-i_s}.$$

Man erhält dann folgende Gleichung:

$$r_{s_n} - r = \left(1 - \frac{a_{s_1}}{1-i_s}\right) \left(1 - \frac{a_{s_2}}{1-i_s}\right) \left(1 - \frac{a_{s_3}}{1-i_s}\right) \dots \\ \dots \left(1 - \frac{a_{s_n}}{1-i_s}\right) (r_s - r).$$

Wird  $\frac{a_s}{1-i_s} = b_s$  gesetzt und ist

$$1 - b_s = \sqrt[n]{(1-b_{s_1})(1-b_{s_2})(1-b_{s_3}) \dots (1-b_{s_n})},$$

so erhält man

$$r_{s_n} - r = (1-b_s)^n (r_s - r). \quad (68)$$

Machen die Ein- und Auswanderung einen Anteil  $p_s$  der Bevölkerung aus, so ist

$$a_s = \frac{p_s}{i_s} \quad \therefore b_s = \frac{p_s}{i_s(1-i_s)}.$$

Die Wahrscheinlichkeit für eine Kreuzung zwischen dem Isolat und der übrigen Bevölkerung ist bei Panmixie  $2 i_s (1-i_s)$ . Da wir annehmen, dass Auswanderung und Einwanderung gleich stark sind, wird die Hälfte der Eheschliessungen innerhalb des Isolates und die Hälfte in der Population stattfinden. Bei Panmixie ist also die Proportion der ein- und ausgewanderten Personen  $i_s (1-i_s)$ . Die wirkliche Proportion ist  $p$  und aus dem Ausdruck geht deswegen hervor, dass  $b_s$  der Anteil ist, welchen die Einwandernden von der

Einwanderung ausmachen, die man bei Panmixie erwarten würde. Findet keine Einwanderung statt, ist  $b_s = 0$ . Und bei Panmixie ist  $b_s = 1$ . Bezeichnet man die Abweichung von 1 mit  $\beta_s$  und ist also  $\beta_s = 1 - b_s$ , so erhalten wir ein direktes Mass für die Stärke der Isolierung,  $\beta_s$  ist also ein Mass für die Isolierung, ein *Isolierungsfaktor*. Man kann die Formel also auf folgende Weise schreiben:

$$r_{s_n} - r = (\beta_s)^n (r_s - r). \quad (69)$$

Für andere Isolate können analoge Formeln aufgestellt werden und wir erhalten deswegen folgenden Ausdruck für die Abweichung der Isolate von dem Anlagegehalt der Population nach  $n$  Einwanderungen:

$$\sigma_n^2 = r_1 (\beta_1)^{2n} (r_1 - r)^2 + r_2 (\beta_2)^{2n} (r_2 - r)^2 + \dots + r_s (\beta_s)^{2n} (r_s - r)^2. \quad (70)$$

Tauscht man die Isolierungsfaktoren  $\beta_1, \beta_2 \dots \beta_s$  gegen einen durchschnittlichen Faktor  $\beta$  aus, so wird folgende Formel erhalten:

$$\sigma_n^2 = (\beta)^{2n} \sigma^2 \quad (71)$$

oder

$$\sigma_n = (\beta)^n \sigma.$$

Hieraus wird erhalten:

$$n = \frac{\log \frac{\sigma_n}{\sigma}}{\log \beta}. \quad (72)$$

Mit Hilfe dieser Formel kann man berechnen, wie viele Generationen vergangen sind, und zwar bei einer gewissen Intensität der Isolierung, bevor ein bestimmter Grad des Ausgleichs zwischen den Isolaten stattgefunden hat. Man kann z. B. die Forderung aufstellen, dass der Ausgleich eine Verminderung der Standardabweichung um  $\frac{1}{10}$  oder  $\frac{1}{20}$  bedeuten soll. Es zeigt sich, dass falls der Isolierungsfaktor 0.5 ist, man eine Homogenität der Population erreicht hat, die bedeutet, dass die Standarddeviation nach 3 Generationen auf  $\frac{1}{10}$  und nach 4 Generationen auf  $\frac{1}{20}$  abgenommen hat. Ist der Isolierungsfaktor 0.9, so sind die entsprechenden Werte 22 und 29 Generationen. Vgl. Tabelle 18.

TABELLE 18.

Geschwindigkeit des Ausgleiches der Gametenproportionen einer Population bei verschiedenen Werten des Isolierungsfaktors.

Wenn eine Population als homogen betrachtet wird bei einer Verringerung der Standarddeviation zu	tritt nach folgender Zahl von Generationen Homogenität ein, falls der Isolierungsfaktor =								
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
$\frac{1}{5}$	1	1	1	2	2	3	5	7	15
$\frac{1}{10}$	1	1	2	3	3	5	6	10	22
$\frac{1}{20}$	1	2	3	3	4	6	8	13	29

### Auflösung der Isolate bei Polyhybridität.

Wenn die Isolatgrenzen vollständig aufgehoben werden, wird in einer einzigen Generation ein Gleichgewicht erhalten, wenn es sich um monohybride Eigenschaften handelt; hingegen geht die Verdünnung nicht ebenso schnell vor sich, wenn es sich um polyhybride Eigenschaften handelt. Um dies zu untersuchen, nehmen wir an, dass eine Vermischung von 2 Populationen, in welchen eine dihybride Eigenschaft in unterschiedlicher Frequenz vorkommt, stattfindet. Vgl. WAHLUND 1928.

Wir bezeichnen bei dialleler Dihybridität die beiden rezessiven Gene mit  $R_1$  und  $R_2$ , welche den dominanten Genen  $D_1$  und  $D_2$  entsprechen. Es wird angenommen, dass die ersteren in einer Population mit der Frequenz  $r_1$  und  $r_2$ , die letzteren mit einer Frequenz von  $d_1$  und  $d_2$  vorkommen, wobei  $r_1 + d_1 = 1$  und  $r_2 + d_2 = 1$ . Die Gene werden zu den Gameten  $R_1 R_2$ ,  $R_1 D_2$  und  $D_1 R_2$  und  $D_1 D_2$  zusammengesetzt und deren Frequenzen mit  $(r_1 r_2)$ ,  $(r_1 d_2)$ ,  $(d_1 r_2)$  bzw.  $(d_1 d_2)$  bezeichnet, wobei  $(r_1 r_2) + (r_1 d_2) + (d_1 r_2) + (d_1 d_2) = 1$ . Würden die Gene zufallsbedingt kombiniert sein, müsste  $(r_1 r_2) = r_1 r_2$ ,  $(r_1 d_2) = r_1 d_2$  u. s. w. sein. Wir nehmen indessen an, dass durch Einmischung in die Population diese Zufallslage gestört wird, so dass

$$(r_1 r_2) = r_1 r_2 + \mathcal{A}_{R_1 R_2}$$

$$(r_1 d_2) = r_1 d_2 + \mathcal{A}_{R_1 D_2}$$

$$(d_1 r_2) = d_1 r_2 + \mathcal{A}_{D_1 R_2}$$

$$(d_1 d_2) = d_1 d_2 + \mathcal{A}_{D_1 D_2}$$

Durch paarweise Summierung dieser Gleichungen und weil  $r_1 + d_1 = 1$  und  $r_2 + d_2 = 1$  erhält man:

$$\mathcal{A}_{R_1 R_2} = -\mathcal{A}_{R_1 D_2} = -\mathcal{A}_{D_1 R_2} = \mathcal{A}_{D_1 D_2}.$$

Die Formeln können also auf folgende Weise geschrieben werden:

$$(r_1 r_2) = r_1 r_2 + \mathcal{A}_{R_1 R_2}$$

$$(r_1 d_2) = d_1 r_2 - \mathcal{A}_{R_1 R_2}$$

$$(d_1 r_2) = d_1 r_2 - \mathcal{A}_{R_1 R_2}$$

$$(d_1 d_2) = d_1 d_2 + \mathcal{A}_{R_1 R_2}$$

Wir nehmen nun an, dass zufällige Kreuzungen in unserer Population stattfinden. Bei einer bestimmten einzigen Kreuzung kann ein Gamet, z. B.  $R_1 R_2$  *ungebrochen* vererbt werden. Darunter verstehen wir, dass beide Gene von einem der Eltern erhalten werden. In solchen Fällen wird die Gametenfrequenz,  $(r_1 r_2)$  unverändert ausfallen und  $(r_1 r_2) = r_1 r_2 + \mathcal{A}_{R_1 R_2}$ . Ebenso oft kommt es vor, dass Gameten *gebrochen* werden, so dass ein Gen von jedem der Eltern erhalten wird. Die Gene treffen hierbei zufallsbedingt mit der Frequenz  $r_1 r_2$  zusammen.

Nach einer Kreuzung wird also die Frequenz für den Gameten  $R_1 R_2$ , bezeichnet mit  $(r_1 r_2)_1$  aus folgender Formel erhalten:

$$(r_1 r_2)_1 = r_1 r_2 + \frac{1}{2} \mathcal{A}_{R_1 R_2}.$$

Nach einer bestimmten Anzahl,  $n$ , Kreuzungen ( $n$ -te Generation) wird, wie leicht einzusehen ist, die Frequenz für den Gameten  $R_1 R_2$  bezeichnet mit  $(r_1 r_2)_n$  durch folgende Formel bestimmt:

$$(r_1 r_2)_n = r_1 r_2 + \frac{1}{2^n} \mathcal{A}_{R_1 R_2}.$$

Werden nun die Formeln darauf ausgedehnt, für alle die vier Gametenarten zu gelten, so wird mit analogen Bezeichnungen erhalten

$$(r_1 r_2)_n = r_1 r_2 + \frac{1}{2^n} \mathcal{A}_{R_1 R_2} = \frac{1}{2^n} (r_1 r_2) + \frac{2^n - 1}{2^n} r_1 r_2 \quad (73 a)$$

$$(r_1 d_2)_n = r_1 d_2 - \frac{1}{2^n} \mathcal{A}_{R_1 R_2} = \frac{1}{2^n} (r_1 d_2) + \frac{2^n - 1}{2^n} r_1 d_2 \quad (73 b)$$

$$(d_1 r_2)_n = d_1 r_2 - \frac{1}{2^n} \mathcal{A}_{R_1 R_2} = \frac{1}{2^n} (d_1 r_2) + \frac{2^n - 1}{2^n} d_1 r_2 \quad (73 c)$$

$$(d_1 d_2)_n = d_1 d_2 + \frac{1}{2^n} \mathcal{A}_{R_1 R_2} = \frac{1}{2^n} (d_1 d_2) + \frac{2^n - 1}{2^n} d_1 d_2. \quad (73 d)$$

Die oben stehenden Formeln geben an die Hand, dass falls in eine Population fremde dihybride Vererbungsmomente eingemischt werden, man nicht nur den unmittelbaren Effekt erhält, den die eingemischten Elemente direkt auf die Zusammensetzung der Bevölkerung ausüben. Es kommt ein sekundärer Effekt hinzu, dessen Wirkungen sich über Generationen erstrecken. Die Population wird nach und nach gegen die Grenzlage  $[(r_1 r_2) \rightarrow r_1 r_2, (r_1 d_2) \rightarrow r_1 d_2 \text{ u. s. w.}]$  verändert, wenn die Gameten in zufallsbedingten Kombinationen und mit zufallsbedingten erwarteten Frequenzen vorkommen.

Diese Entwicklung geht ziemlich schnell vor sich, wenigstens wenn es sich um Vererbungsverhältnisse eines weniger komplizierten Typus handelt. Ich verweise auf folgende Tabelle 19.

Bei polyhybrider Vererbung verbleibt also eine Population heterogen, eine Zeit lang auch bei Panmixie. Nach einer Einmischung fremder Vererbungselemente entwickelt sie sich nur allmählich in Richtung einer vollständigen Homogenität, also gegen die Gleichgewichtslage, in welcher die Gene nicht nur zufallsbedingt innerhalb der Population, sondern auch in zufallsbedingten Kombinationen auftreten.

Wie soll man nun eine Verschiebung der oben beschriebenen Art nachweisen können? Eine Möglichkeit besteht darin, die Population in Gruppen mit verschiedener Abstammung aufzuteilen und diese Gruppen miteinander zu vergleichen. Man trennt die Personen in der Bevölkerung, die von Eingewanderten abstammen, von den Nachkommen der in einem bestimmten Gebiet Einheimischen. In der letzteren Gruppe unterscheidet man Teilgruppen, in welchen die Einmischung in naheliegenden Generationen geschehen ist und Teil-





gruppen, in welchen sie vor langer Zeit stattgefunden hat. Durch die Untersuchung der Frequenz von Eigenschaften innerhalb dieser Gruppen, die auf diese Weise erhalten wurden, kann man Anhaltspunkte für die Beurteilung der Situation erhalten. Dieser Weg ist selbstverständlich sehr mühsam. Eine einfachere Möglichkeit ist die, die Korrelationsverhältnisse zwischen verschiedenen Erbeigenschaften zu untersuchen.



Kommen nun in einer Population verschiedene Rassen vor, so muss dies bedeuten, dass Gruppen von Isolaten eine vom Standpunkt der Vererbung abweichende Beschaffenheit aufweisen. Dies bringt es mit sich, dass eine Korrelation vorliegt. Untersucht man z. B. die Bevölkerung in Amerika, so wird man eine Korrelation zwischen der Hautfarbe, gekräuselttem Haar u. s. w. finden, was darauf beruht, dass in der Population Gruppen von Isolaten, bestehend aus Negern bzw. Weissen vorhanden sind. Findet man in einer Population hingegen, dass keine Korrelation zwischen den physischen Eigenschaften, denen aus Gesichtspunkten der Rasse Bedeutung zugeschrieben werden, vorliegen, so bedeutet dies, dass auf Grund der Aufhebung der Isolate, die Population homogen geworden ist, so dass es selbstverständlich dann nicht mehr berechtigt ist, von Rassenunterschieden innerhalb des Volkes zu sprechen.

Auf Grund dessen, dass eine Aufhebung von Isolaten stattfindet, ist es zu erwarten, dass die Rassenunterschiede innerhalb eines Volkes geringer werden. Es ist zweifelsohne so, dass in zahlreichen europäischen Ländern eine Einwanderung vorgekommen ist, welche die Bildung von Isolaten, oft von sozialen Isolaten in Form einer Oberklasse von Eroberern, bewirkt hat; auf Grund der Aufhebung der Isolate haben die Unterschiede, die innerhalb des Volkes entstanden sind, abgenommen und sind dann verschwunden.

Werden zwei Bevölkerungen miteinander vermischt, so muss es eine kürzere oder längere Zeit dauern, bevor die Gleichgewichtslage, die man bei Panmixie zu erwarten hat, erreicht wird. Auch bei freier Kreuzung dauert es mehrere Generationen, ehe nicht nur für polyhybride Eigenschaften sondern auch für Kombinationen von monohybriden Eigenschaften eine Gleichgewichtslage erreicht wird.

Wählen wir ein schematisches Beispiel. (Vgl. WAHLUND 1932.) Eine bestimmte „reine Rasse“  $A$  soll Träger von zwei monohybriden erblichen Eigenschaften I und II sein, welche durch die Gene  $A_1$  und  $A_2$  bedingt werden. Eine zweite „reine Rasse“  $a$  besitzt diese beiden Eigenschaften nicht oder, wie man die Sachlage ausdrücken kann, ist Träger von Eigenschaften Nicht-I und Nicht-II mit der Erbanlage  $a_1$  und  $a_2$ . Würden nun diese beiden Rassen miteinander innerhalb eines Gebietes vorkommen, und zwar die Rasse  $A$  in der Frequenz  $P$ , die Rasse  $a$  in der Frequenz  $p$ , ( $P + p = 1$ ), so werden folgende Beziehungen erhalten, die für die in dem Gebiet vorhandene Population gelten:

$$\begin{array}{ll}
(A_1) = P & (A_1 A_2) = P \\
(a_1) = p & (A_1 a_2) = 0 \\
(A_2) = P & (a_1 A_2) = 0 \\
(a_2) = p & (a_1 a_2) = p
\end{array}$$

$(A_1)$ ,  $(A_2)$  und  $(A_1 A_2)$  bezeichnen die Anlagefrequenzen, bzw. die Frequenz der Anlagekombination, die nur bei der Rasse  $A$  vorkommt und in der Population mit ihrer Frequenz  $P$  repräsentiert wird. Die Frequenzen  $(a_1)$ ,  $(a_2)$  und  $(a_1 a_2)$  beziehen sich auf die Rasse  $a$  und haben also die Frequenz  $p$ . Die Kombinationen  $(A_1 a_2)$  und  $(a_1 A_2)$  sind in der Population nicht repräsentiert.

Soll nun eine Untersuchung über die Korrelation zwischen den Eigenschaften I und II angestellt werden, so wird der Korrelationskoeffizient  $R$  wie folgt erhalten:

$$R = \frac{(A_1 A_2) - (A_1) \cdot (A_2)}{\sqrt{(A_1) \cdot (a_1) \cdot (A_2) \cdot (a_2)}} = 1.$$

In der ersten Kreuzungsgeneration,  $F_1$ -Generation, werden folgende Zygoten erhalten:

$$\begin{array}{l}
(A_1 A_1 A_2 A_2) \text{ mit der Frequenz } P^2 \\
(A_1 a_1 A_2 a_2) \text{ mit der Frequenz } 2 Pp \\
(a_1 a_1 a_2 a_2) \text{ mit der Frequenz } p^2
\end{array}$$

Diese Zygoten veranlassen Anlagekombinationen, deren Frequenzen mit  $(A_1 A_2)_1$ ,  $(A_1 a_2)_1$ ,  $(a_1 A_2)_1$  und  $(a_1 a_2)_1$  bezeichnet werden. Man erhält leicht:

$$(A_1 A_2)_1 = P^2 + \frac{1}{2} Pp$$

$$(A_1 a_2)_1 = \frac{1}{2} Pp$$

$$(a_1 A_2)_1 = \frac{1}{2} Pp$$

$$(a_1 a_2)_1 = p^2 + \frac{1}{2} Pp$$

Eine Untersuchung der Korrelation zwischen den Eigenschaften I und II ergibt nun

$$R_1 = \frac{(A_1 A_2)_1 - (A_1) \cdot (A_2)}{\sqrt{(A_1) \cdot (a_1) \cdot (A_2) \cdot (a_2)}} = \frac{1}{2}$$

Von der Ausgangsgeneration zur  $F_1$ -Generation hat die Korrelation von 1 auf  $\frac{1}{2}$  abgenommen.

Verändern wir nun unsere ursprüngliche Annahme um Reinrassigkeit. Wir bezeichnen mit  $A$  und  $a$  zwei vom Standpunkt der Vererbung aus verschiedenartige Populationen. Auf Grund der Heterogenität im Hinblick auf die Vererbung würde dann in der Ausgangsgeneration eine Korrelation zwischen den Eigenschaften I und II entstehen, wobei der Korrelationskoeffizient  $R$  kleiner als 1 ist.

Es kann leicht gezeigt werden, dass in diesem Falle der Korrelationskoeffizient, bezeichnet mit  $R_1$  nach einer Kreuzung ( $F_1$ -Generation) um die Hälfte seines ursprünglichen Wertes abnimmt. Wir haben also:

$$R_1 = \frac{1}{2} R.$$

Setzt die Rassenmischung fort, so wird in der  $n$ -ten Generation eine „Restkorrelation“ von

$$R_n = \frac{1}{2^n} R \quad (74)$$

erhalten.

Der Mechanismus für die Korrelationsveränderungen ist analog mit dem für den Entwicklungsgang bei polyhybriden Eigenschaften. Auf Grund dessen kann man beim Studium der Korrelation zwischen erblichen Eigenschaften feststellen, inwieweit die Bevölkerung vom Standpunkt der Vererbung aus als homogen anzusehen ist oder inwieweit sie durch ältere Einmischungen heterogen und sich auf dem Wege der Entwicklung zu einer Gleichgewichtslage befindet, in welcher die Anlagen zufallsbedingt und in zufallsbedingten Kombinationen vorkommen.

Es ist also deutlich, dass falls man in einer Bevölkerung keinerlei Korrelation zwischen den Eigenschaften findet, man die Bevölkerung als in Hinsicht auf die Vererbung homogen bezeichnen muss. Es besteht dann kein Anlass über verschiedene Rassenelemente in der Bevölkerung zu sprechen. Handelt es sich um eine praktische Untersuchung, ist es indessen klar, dass man hierbei nicht die Korrelationen zwischen beliebigen Eigenschaften anwenden kann. Es ist klar, dass man z. B. nicht die Korrelation zwischen Beinlänge und Körperlänge anwenden kann, da die Beinlänge in der Körperlänge enthalten ist. Man kann auch nicht die Korrelation zwischen Beinlänge und Arm-



eine höhere Frequenz haben unter den Nachkommen von Verwandtschaftsesehen näheren Grades als unter Individuen, die von nicht verwandten Individuen abstammen. Damit in Verbindung steht schliesslich die Erscheinung, dass, wenn man Material bezüglich selten vorkommender Eigenschaftsträger einsammelt, man diese bei zufälliger Auswahl vor allem aus kleinen Isolaten holt, in welchen von Mutationen bedingte Eigenschaften besonders grosse Aussicht haben sich geltend zu machen, weswegen man auch aus diesem Grunde theoretisch betrachtet eine Korrelation zwischen solchen Eigenschaften erwarten dürfte. Um das letztere zu verdeutlichen, nehmen wir an, dass eine Mutation mit der Frequenz  $10^5$  auftritt. In einem grossen Isolat, einer Millionenstadt z. B., ist die Aussicht, dass die Anlage in doppelter Dosis zusammenkommen wird, verhältnismässig gering. Wenn eine Mutation dagegen in einem der kleinen Isolate, das einige hundert Individuen umfasst, eintritt, so sind, wie wir schon betont haben, die Aussichten, dass die Mutation so nach und nach manifeste Eigenschaftsträger nach sich ziehen wird, weit grösser. Aus diesem Grunde hat man also eine Korrelation zwischen Eigenschaftsträgern dieses Types zu erwarten.

### Isolat und Rassenunterschiede.

Weiter oben wurde bereits hervorgehoben, dass man, um sich über Rassenunterschiede eingehender orientieren zu können, den Genotypus der verschiedenen Individuen in den beiden Isolatgruppen kennen muss, die man miteinander vergleichen will. Wir haben ferner die Methoden angedeutet, die bei einer Analyse der Verschiedenheiten zwischen den Isolaten zur Anwendung kommen können. Für die Anwendung dieser Formeln sind aber Aufklärungen erforderlich, die gegenwärtig nur schwer erhalten werden können. Es ist unter allen Umständen erwünscht, ein Mass für die Grösse evt. Unterschiede zwischen den Isolatgruppen zu erhalten. Im grossen und ganzen hat man sich bisher auf dem Gebiete der Anthropologie damit begnügt, mit Hilfe von statistischen Methoden das Vorhandensein von Unterschieden feststellen zu wollen. Es bedeutet einen Fortschritt, dass man bezüglich Rassenunterschiede versucht, die subjektive Beurteilung zu verlassen und sichergestellte Unterschiede zu erreichen. Man muss aber einen Schritt weitergehen und versuchen, eine Vor-

stellung über die Grösse der Unterschiede auf eine solche Art zu erlangen, dass man Vergleiche zwischen mehreren Gruppen anstellen und so die Unterschiede in Beziehung zu der transgredienten Variation setzen kann, die zwischen den Gruppen vorliegt. Die Methoden, die hier angegeben werden, können selbstverständlich auch angewandt werden, wenn es sich darum handelt, ein Mass für die Unterschiede zwischen anderen Materialgruppen und Eigenschaften als Rassenunterschieden und Rassengruppen zu erhalten. (Vgl. DAHLBERG 1941).

Eine Möglichkeit besteht darin, die Rassenunterschiede mit Hilfe der Frequenz zu messen, mit welcher eine sichere Diagnose im Einzelfalle gestellt werden kann. Im Prinzip ist die Art des Vorgehens folgende. Man untersucht zuerst die beiden Populationen, die verglichen werden sollen, so dass man eine Vorstellung über die Frequenz der verschiedenen Eigenschaften und ihre Variabilität erhält. Hernach untersucht man, in welchem Ausmasse mit Hilfe einzelner Eigenschaften oder Kombinationen von Eigenschaften die Personen in die einzelnen Populationen, zu denen sie gehören, eingereiht werden können. Man stellt sich mit anderen Worten vor, dass man die Individuen auf einem Marktplatz miteinander vermischt. Dann geht man z. B. von der Körperlänge aus. Man kann dann bestimmte Individuen auf Grund dieser Eigenschaft herausortieren. Einige weisen eine allzu geringe Körperlänge auf, um der einen Gruppe angehören zu können, oder sie sind zu lang, um der zweiten Gruppe anzugehören. Hernach untersucht man den Kopfindex auf dieselbe Weise u. s. w. Schliesslich untersucht man die Kombinationen von Eigenschaften. Eine bestimmte Person kann auf Grund ihrer Körperlänge oder ihres Kopfindexes zu beiden Gruppen gezählt werden, die Kombinationen aber, die sie aufweist, kommt aber vielleicht nur in der einen Gruppe vor. Handelt es sich um qualitative Eigenschaften wie die Augenfarbe, so kann man keine empirische Sortierung nach diesen Linien ausführen, wenn es sich nur um Frequenzunterschiede zwischen den Gruppen handelt; man kann aber mit Hilfe dieser Unterschiede berechnen, welches Ergebnis man auf Grund der Unterschiede erhalten würde.

Will man zwei Populationen ( $P'$  und  $P''$ ) im Hinblick auf eine quantitativ messbare Eigenschaft  $A$  ( $A'$  und  $A''$ ), die eine normale Verteilung hat, vergleichen, so kann man den Wert für jede Person in Abweichungen von den zusammengeschlagenen Mittelwerten



beider Populationen ausdrücken und die Abweichung in der Standarddeviation für die beiden Populationen angeben, zu welchen die entsprechenden Personen gehören. Den Mittelwert für die zusammengeschlagene Population bezeichnen wir wie folgt:

$$\frac{M'_A + M''_A}{2}$$

In der Population  $P'$  wird für die Eigenschaft  $A'$  in einer Serie von  $n$  Individuen folgender Mittelwert erhalten:

$$M' = \frac{1}{n'} \sum \frac{A'_i - \frac{M'_A + M''_A}{2}}{\sigma'_A} = \frac{M'_A - M''_A}{2 \sigma'_A}$$

Die Standarddeviation (die gleich 1 gesetzt wird) ist dann

$$\sigma'^2 = E \left[ \frac{A'_i - \frac{M'_A + M''_A}{2}}{\sigma'_A} \right]^2 = 1$$

Für die Population  $P''$  erhalten wir folgenden Mittelwert:

$$M'' = \frac{1}{n''} \sum \frac{A''_i - \frac{M'_A + M''_A}{2}}{\sigma''_A} = \frac{M''_A - M'_A}{2 \sigma''_A}$$

Die Standarddeviation wird auch jetzt = 1 gesetzt.

Haben die Populationen verschiedene Mittelwerte, so werden selbstverständlich die beiden neuen Mittelwerte ein positives bzw. negatives Vorzeichen haben. Sind die Standarddeviationen in den beiden Populationen gleich gross, so werden die Mittelwerte denselben zahlenmässigen Wert haben.

Kombinieren wir  $N$  Eigenschaften, so erhält man für die beiden Populationen folgende Mittelwerte:

$$M'_s = \sum_{i=A}^N \frac{M'_i - M''_i}{2 \sigma'_i} \quad (75 a)$$

$$M''_s = \sum_{i=A}^N \frac{M''_i - M'_i}{2 \sigma''_i} \quad (75 b)$$

Sind die Standarddeviationen für beide Eigenschaften 1, so erhält man bei der Kombination von  $N$  Eigenschaften die Standarddeviation  $\sqrt{N}$ , insoweit keine Korrelation zwischen den Eigenschaften vorliegt.

Stehen die Eigenschaften hingegen in einer Korrelation, so erhält man eine grössere Dispersion. Ist die durchschnittliche Korrelation zwischen den Eigenschaften in der Population  $P'$  und  $P''$   $r'$  und  $r''$ , so erhält man folgende Standarddeviationen:

$$\sigma'^2 = N + N(N-1)r', \quad (76 a)$$

$$\sigma''^2 = N + N(N-1)r''. \quad (76 b)$$

Bei der Kombination von Eigenschaften berücksichtigt man selbstverständlich nicht, dass die Eigenschaften in entgegengesetzter Richtung voneinander abweichen können. Man summiert sie ohne Rücksicht auf das Vorzeichen und lässt die erste Eigenschaft das Vorzeichen bestimmen.

Aus den Formeln ergibt sich, dass falls zwischen den Populationen kleine Unterschiede vorliegen, man durch Untersuchung ausreichend vieler Eigenschaften und unter Benutzung der Kombinationen zwischen diesen schliesslich eine sichere Diagnose in jedem Einzelfall erreichen kann. Eine Voraussetzung hierfür ist aber, dass keine Korrelation vorliegt. Ist die Korrelation stark, so gewinnt man nur wenig durch die Untersuchung einer weiteren Eigenschaft. Ist die Korrelation schwach, so gewinnt man anfänglich eine Erhöhung der Diagnosemöglichkeiten, nachdem man aber eine mässige Anzahl von Eigenschaften benutzt hat, wird der Gewinn unbedeutend. Nimmt man eine bestimmte durchschnittliche Verschiebung des Mittelwertes für jede neue Eigenschaft an, so kann man die kleinste Anzahl von Eigenschaften berechnen, die untersucht werden muss, damit die beiden Verteilungskurven, unter der Voraussetzung, dass die Variationsbreite mit  $6\sigma$  angenommen wird, völlig voneinander getrennt werden. Bezeichnet man die durchschnittliche Verschiebung für jede Eigenschaft mit  $a$  und die Anzahl der Eigenschaften mit  $N$ , sowie die durchschnittliche Korrelation zwischen diesen mit  $r$ , so kann man die kleinste Anzahl von Eigenschaften berechnen, die erforderlich ist, und zwar aus folgender Formel:

$$N = \frac{9(1-r)}{a^2 - 9r} \quad (77)$$



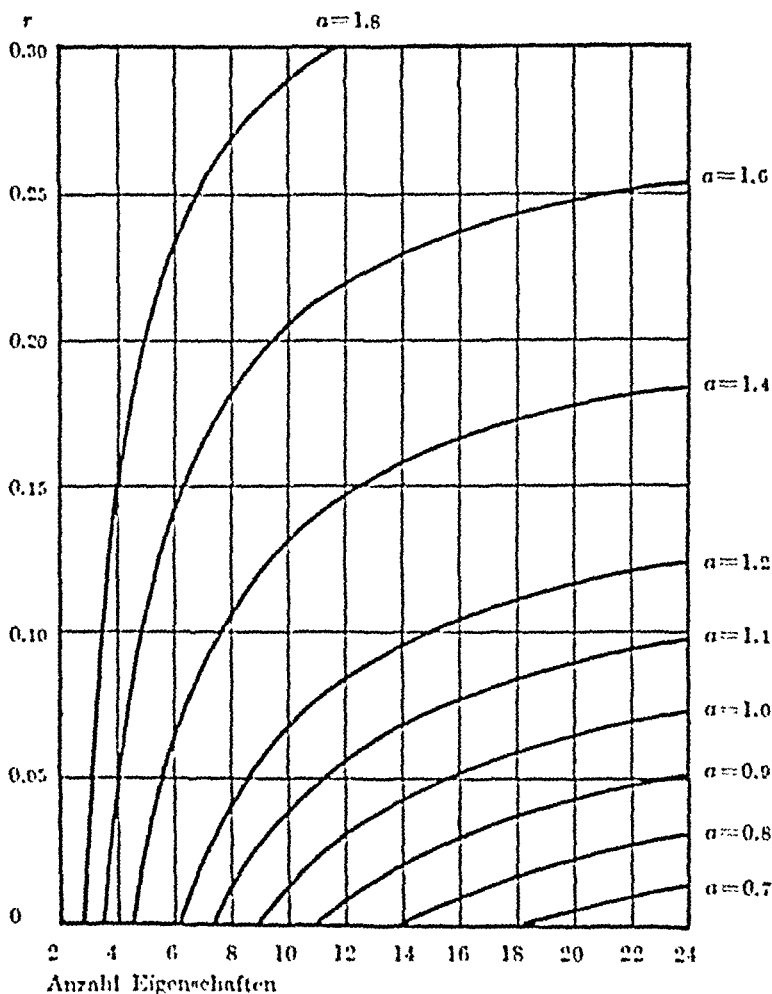


Abb. 22. Kleinste Anzahl Eigenschaften, die erforderlich ist, um jedes Individuum voneinander zu unterscheiden bei verschiedenen Graden durchschnittlicher Korrelation ( $r$ ) und durchschnittlicher Differenz zwischen den Mittelwerten ( $a$ ). Vgl. Formel 77.

schaften benutzt. Man findet, dass auch bei einer sehr schwachen Korrelation der Gewinn, den man durch die Erweiterung der Untersuchung mit einer weiteren Eigenschaft erhält, verhältnismässig klein ist, wenn man sich einer Zahl von etwa 10 Eigenschaften nähert.

Mit Hilfe der angegebenen Methode kann man also eine Vorstellung darüber erhalten, wie gross der Rassenunterschied ist. Man erhält einen Wert, für die Frequenz, mit der man eine sichere Rassen-diagnose stellen kann; hierbei kann man einen Vergleich zwischen



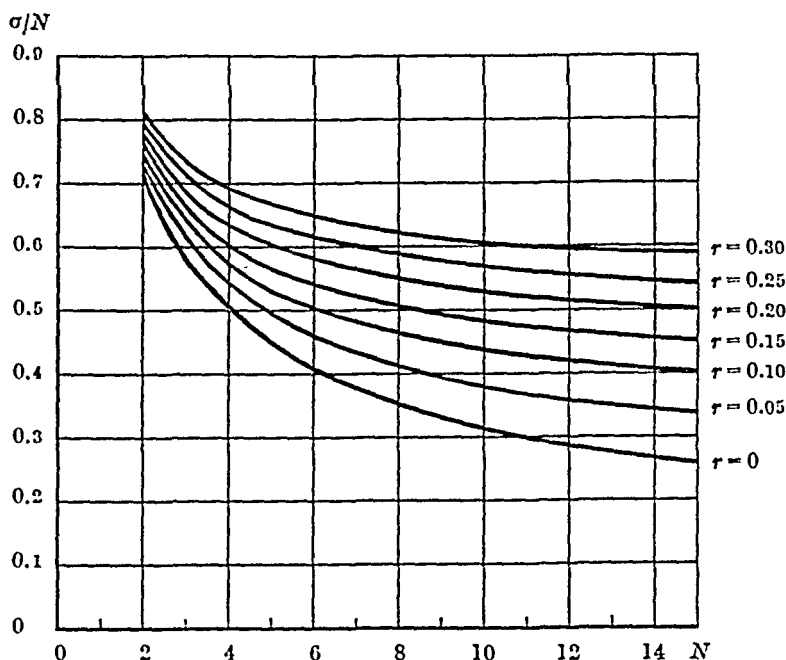


Abb. 23. Der Quotient  $\frac{\sigma}{N}$  bei verschiedenen Graden der Korrelation und verschiedener Anzahl beobachteter Eigenschaften.

wurde, wurde vom Verf. (DAHLBERG 1941) angegeben und wurde zur Bestimmung von Rassenunterschieden zwischen Schweden und Lappländern angewandt. Die beiden Gruppen weisen sehr grosse Unterschiede auf und es zeigt sich, dass bei Anwendung von drei Eigenschaften, nämlich Körperlänge, Kopfindex und morphologischer Gesichtsindex, eine sichere Diagnose auf Lappländer in 57 % gestellt werden kann. Nimmt man die beste Aufteilung des Materials vor, so erhält man 93 % Lappländer und 7 % Schweden. Der Rassenindex ist mit anderen Worten 0.86.

Bereits WEINBERG (1909) und PHILIPTSCHENKO (1924) haben die Frage der Einmischung in eine Population behandelt und gezeigt, dass für polyhybride Eigenschaften erst nach mehreren Generationen eine zufallsbedingte Verteilung der Anlagen erreicht wird. Der Isolatbegriff wurde für menschliche Populationen von WAHLUND (1928) aufgestellt, der Methoden für die Berechnung der Abweichungen angegeben hat, welche die Isolatgrenzen von der Panmixie verursachen sowie ausserdem generelle Formeln für die Bedeutung der Isolat-



## Mutation, Selektion und Isolat.

Wenn in einem Isolat eine Mutation entsteht, kann sie nicht in der ersten Generation Anlass einer Selektion werden (insoweit sie nicht einfach dominant ist). Sie kann aber durch einen Zufall verschwinden, sie kann aber auch durch einen Zufall in die nächste Generation herüberkommen. Im letzteren Fall ist sie immer noch selektionsfrei und ebenso verhält es sich auch in der folgenden Generation, da beim Menschen keine Geschwisterehen vorkommen. Auch in der dritten Generation ist sie praktisch genommen selektionsfrei, da Ehen zwischen Geschwistern der Eltern und Kindern von Geschwistern beim Menschen eine ausserordentlich niedrige Frequenz haben. Erst in der vierten Generation nach einer Vetternehe kann sie Gegenstand einer Selektion werden. Die Anlagen können dann in homozygoter Form zusammentreffen. Indessen muss erwähnt werden, dass die Mutation möglicherweise auch bei einer Vetternnehe in der fünften Generation nicht Gegenstand einer Selektion zu sein braucht, falls die Vettern und Basen, die in der vierten Generation Heterozygoten sind, dasselbe Geschlecht besitzen. Sind zwei Heterozygoten vorhanden, ist ja die Wahrscheinlichkeit hierfür  $\frac{1}{2}$ , und sind drei Heterozygoten vorhanden, ist die Wahrscheinlichkeit  $\frac{1}{4}$ . Dies alles bedeutet, dass Mutationen anfänglich frei von Selektion bleiben. Erst nachdem die Heterozygotie ein gewisses Ausmass erreicht hat, treten Eigenschaftsträger auf, die Gegenstand einer Selektion werden.

Ist die Selektion absolut, so dass jeder Eigenschaftsträger daran gehindert wird sich fortzupflanzen, wird eine Gleichgewichtslage bei einer *kleinsten Heterozygotenfrequenz* eintreten, deren Lage durch die Mutationsfrequenz bestimmt wird. Nehmen wir eine Population an, in welcher die Anlage nicht vorhanden ist, so wird bei einer bestimmten Mutationsfrequenz und absoluter Selektion die Heterozygotenfrequenz langsam bis zu einer Gleichgewichtslage zunehmen. Nehmen wir an, dass die Mutationen aufhören nachdem diese Lage



erreicht ist, so wird die Heterozygotenfrequenz langsam abnehmen und sich asymptotisch 0 nähern, obwohl dies selbstverständlich äusserst langsam vorsichgeht.

Die Lage der kleinsten Heterozygotengrenze beruht nun nicht nur auf der Mutationsfrequenz, sondern auch auf der Intensität der Selektion, sowie auf den Prozessen und Besonderheiten innerhalb der Population, nämlich der Isolatgrösse, der Gattenwahl und den Verwandtschaftsehen. Schliesslich ist selbstverständlich die kleinste Heterozygotengrenze verschieden bei verschiedenen Vererbungsmechanismen.

Nehmen wir an, dass die Gleichgewichtslage bei einer Frequenz der Eigenschaftsträger von  $1:N$  vorhanden ist. Ist die Eigenschaft *monohybrid-rezessiv* und hat die Anlage die Frequenz  $r$ , so ist

$$r^2 = \frac{1}{N}.$$

Deswegen ist  $r = \frac{1}{\sqrt{N}}$ . Die Frequenz der Heterozygoten,  $2rd$  ist dann:

$$2rd = 2 \cdot \frac{1}{\sqrt{N}} \cdot \left(1 - \frac{1}{\sqrt{N}}\right) \quad (78)$$

Ist  $N$  eine grosse Zahl, so wird der Ausdruck approximativ:

$$2rd = \frac{2}{\sqrt{N}}$$

Bei *polyhybrider Rezessivität*, wenn die verschiedenen Anlagen die Frequenzen  $r_1, r_2, r_3 \dots r_n$  haben, besteht eine Gleichgewichtslage bei

$$r_1^2 \cdot r_2^2 \cdot r_3^2 \dots r_n^2 = \frac{1}{N}$$

Ist  $r_1 = r_2 = r_3 = \dots r_n = r$ , also jedes  $d_x = d$  so wird erhalten:

$$r^{2n} = \frac{1}{N} \text{ und } r = \sqrt[2n]{\frac{1}{N}}$$

Die Frequenz der Heterozygoten wird dann:

$$1 - r^{2n} - d^{2n} = 1 - \frac{1}{N} - \left(1 - \frac{1}{\sqrt[2n]{N}}\right)^{2n} \quad (79)$$

Ist  $N$  zahlenmässig gross,  $n$  aber nicht, so wird

$$1 - r^{2n} - d^{2n} \approx \frac{2n}{\sqrt{N}}$$

Bei *polyhybrider Dominanz*, wenn die dominante Anlage die Frequenzen  $d_1, d_2, d_3 \dots d_n$  und die rezessive Anlage die Frequenzen  $r_1, r_2, r_3 \dots r_n$  aufweisen, liegt die Gleichgewichtslage bei:  $(d_1^2 + 2r_1d_1) \cdot (d_2^2 + 2r_2d_2) \dots (d_n^2 + 2r_nd_n) = \frac{1}{N}$

Ist  $d_1 = d_2 = d_3 = \dots d_n$ , so wird erhalten:

$$(d^2 + 2rd)^n = \frac{1}{N} \text{ und} \\ d = 1 - \sqrt[n]{1 - \frac{1}{\sqrt{N}}} \approx \frac{1}{2\sqrt{N}} \quad (80 \text{ a})$$

$$\text{sowie } r = \sqrt[n]{1 - \frac{1}{\sqrt{N}}} \approx 1 - \frac{1}{2\sqrt{N}} \quad (80 \text{ b})$$

Der Heterozytengehalt bei der kleinsten Heterozygotengrenze ist dann

$$1 - (d^2 + 2rd)^n - r^{2n} = 1 - \frac{1}{N} - \left(1 - \frac{1}{\sqrt{N}}\right)^n \approx \frac{n}{\sqrt{N}} \quad (81)$$

Mit Hilfe dieser Formeln kann man, falls man die Frequenz der Eigenschaftsträger kennt, die Gegenstand einer Selektion sind, die Frequenz der Anlagen und die Frequenz der Heterozygoten für die verschiedenen Vererbungsmechanismen berechnen.

Wir haben bereits früher hervorgehoben, dass bei einer bestimmten Mutationsfrequenz und bei einer bestimmten Selektion allmählich eine Gleichgewichtslage eintreten muss, in welcher die Anzahl von Personen, die durch Selektion ausgerottet werden, der Anzahl Mutationen bei monohybrider Dominanz und doppelter Mutationsfrequenz bei monohybrider Rezessivität entspricht. Nehmen wir an, dass aus irgend einem Anlass diese Gleichgewichtslage gestört ist. Wir nehmen an, dass die Anlage die Frequenz  $r$  hat. Die Mutations-

frequenz ist  $\mu$ . Durch Selektion werden  $k$  der Eigenschaftsträger,  $r^2$ , ausgerottet. In dieser Situation kommt es zu einer Verschiebung von Generation zu Generation. Der Unterschied zwischen zwei aufeinander folgenden Generation ist:

$$r_{n+1} - r_n = r_n - kr_n^2 + 2\mu(1 - r_n) - r_n$$

Nachdem sich der Prozess asymptotisch einer Gleichgewichtslage nähert, wird er eine unendlich lange Zeit weitergehen. Man kann dann die Zeit, die eine Generation repräsentiert als unendlich klein ansehen, so dass die Verschiebung zwischen zwei Generationen als die Derivate im Hinblick auf die Zeit für eine Funktion angesehen werden kann, die den Verlauf des Prozesses ausdrückt. Es ist also

$$\frac{dr}{dn} = -kr^2 + 2\mu(1 - r)$$

$$\therefore dn = - \frac{dr}{k \left( r^2 + \frac{2\mu r}{k} - \frac{2\mu}{k} \right)}$$

Durch Integration erhalten wir

$$n = - \frac{1}{k} \int_{r'_0}^r \frac{dr}{r^2 + \frac{2\mu r}{k} - \frac{2\mu}{k}}$$

$$\therefore n = \frac{1}{2\sqrt{2\mu k + \mu^2}} \log \frac{r - r'_2}{r - r'_1} \cdot \frac{r'_0 - r'_1}{r'_0 - r'_2} \quad (82)$$

$$\text{wobei } r'_1 = \frac{\sqrt{2\mu k + \mu^2} - \mu}{k}$$

$$r'_2 = - \frac{\sqrt{2\mu k + \mu^2} + \mu}{k}$$

und  $r'_0$  die Anlagefrequenz ist, die in der Ausgangsgeneration vorhanden ist,  $r'_1$  die Anlagefrequenz in der Gleichgewichtslage und  $r$  ein Wert zwischen diesen beiden ist, für den man die Zeit berechnen will. Man muss sich daran erinnern, dass die Gleichung nicht angewandt werden kann, wenn  $r'_0$  nahe an dem Wert von  $r'_1$  liegt. Wir haben angenommen, dass die Zeit, welche eine Generation repräsentiert

tiert, im Verhältnis zu der Zeit sehr klein ist, die vergeht, bis eine Gleichgewichtslage erreicht wird; für sehr kleine Verschiebungen von der Gleichgewichtslage ist dies nicht der Fall. Man ist dann nicht berechtigt, die diskontinuierliche Funktion als kontinuierlich anzusehen. Im übrigen ergibt sich aus der Gleichung: ist  $r'_0$  von  $r'_1$  verschieden, aber  $r'_1 = r$ , d. h. der gesuchte Wert gleich der Frequenz in der Gleichgewichtslage zwischen Selektion und Mutation, so wird  $n = \infty$ . Dies bedeutet, dass die Gleichgewichtslage erst nach einer unendlich langen Zeit erreicht wird. Es ist dann von Interesse, die Zeit zu berechnen, die vergeht, bevor die Gleichgewichtslage zur Hälfte erreicht wird, d. h. bis die Frequenz erreicht wird, die der Mittelwert des Ausgangswertes und der Frequenz beim Gleichgewicht ist. Nehmen wir an, dass  $r'_0 = cr'_1$  und also

$$r = \frac{1}{2} r'_1 (c + 1) \text{ ist.}$$

Wir erhalten dann

$$\begin{aligned} n &= \frac{1}{2\sqrt{2\mu k + \mu^2}} \log \frac{\frac{1}{2} r'_1 (c + 1) - r'_2}{\frac{1}{2} r'_1 (c + 1) - r'_1} \cdot \frac{cr'_1 - r'_1}{cr'_1 - r'_2} = \\ &= \frac{1}{2\sqrt{2\mu k + \mu^2}} \log \frac{r'_1 (c + 1) - 2r'_2}{cr'_1 - r'_2} \end{aligned} \quad (83)$$

Ist  $k = 1$ , d. h. liegt eine totale Selektion vor, und ist  $\sqrt{2\mu} \ll 1$ , so wird

$$\begin{aligned} r'_1 &\approx \sqrt{2\mu} \text{ und } r'_2 \approx -\sqrt{2\mu} \\ \therefore n &= \frac{1}{2\sqrt{2\mu}} \log \frac{3 + c}{1 + c} \end{aligned} \quad (84 a)$$

ist  $k \neq 1$  und  $\sqrt{\frac{2\mu}{k}} \ll 1$  wird

$$\begin{aligned} r'_1 &= \sqrt{\frac{2\mu}{k}} \text{ und } r'_2 = -\sqrt{\frac{2\mu}{k}} \\ \therefore n &= \frac{1}{2k\sqrt{\frac{2\mu}{k}}} \log \frac{3 + c}{1 + c} \end{aligned} \quad (84 b)$$

Mit Hilfe dieser Ausdrücke können wir die Anzahl von Generationen berechnen, die für eine Verschiebung von einer bestimmten Frequenz der Eigenschaftsträger bis zu einer anderen Frequenz notwendig ist. Wir haben bereits früher hervorgehoben, dass auf Grund der Isolation die Frequenz sehr seltener monohybrider Eigenschaften in einer Reihe von westeuropäischen Ländern auf die Hälfte der Frequenz abgenommen hat, die vor etwa 50 Jahren vorhanden war. Es dauert aber eine unendlich lange Zeit, ehe erneut eine Gleichgewichtslage erreicht wird. Es kann aber von Interesse sein, die Zeit zu berechnen, die vergeht, um den Abstand zwischen der jetzigen Frequenz und der Gleichgewichtslage zu halbieren. Ist die ursprüngliche Frequenz  $r^2 = 2\mu$  und die jetzige Frequenz  $\frac{r^2}{2}$ , so liegt der halbe Abstand bei:

$$r \left( \frac{1}{2\sqrt{2}} + \frac{1}{2} \right) = \sqrt{\mu} \left( \frac{1}{2} + \frac{1}{\sqrt{2}} \right)$$

Wir benötigen ferner einen Wert für die Mutationsfrequenz. HALDANE hat approximative und selbstverständlich äusserst unsichere Berechnungen ausgeführt, die eine Mutationsfrequenz von  $1:10^6$  ergeben. Nehmen wir an, dass eine totale Selektion vorliegt, so finden wir mit Hilfe von Formel 84 a, dass nach 123 Generationen der Unterschied zur Hälfte ausgeglichen wurde, was, falls man mit 25 Jahren pro Generation rechnet, 3000 Jahren entsprechen würde. Die Berechnung gibt eine Vorstellung darüber, wie langsam die Mutationsfrequenz eine Abweichung von der Gleichgewichtslage ausgleicht.

HALDANE (1939 und 1940) hat eine ähnliche Berechnung mit dem Ausgangspunkt ausgeführt, dass in einer Population eine bestimmte Frequenz an Verwandtschaftsehen vorliegt, die grösser ist, als die bei Panmixie zu erwartende, und dass die Gleichgewichtslage, die in dieser Situation vorliegt, dadurch verändert wird, dass sich die Frequenz an Verwandtschaftsehen verändert. Die Berechnungen, die HALDANE ausgeführt hat, ergeben Werte von der gleichen Grössenordnung.

Wir haben weiter oben die Mutationsfrequenz und die Selektion unter der Annahme besprochen, dass die Prozesse in sehr grossen Populationen und während einer langen Zeit vorsichgehen. In Wirklichkeit sind ja die Grössen der Populationen begrenzt, und sie sind ausserdem in Isolate von mässiger Grössenordnung aufgeteilt. Die Frequenz an Anlagen, die durch Mutationen in den Isolaten entstehen,

ist in Wirklichkeit in hohem Grade zufallsbedingt. Entsteht ein Heterozygot auf Grund einer Mutation, so ist die Wahrscheinlichkeit, dass die Anlage verschwinden und in der nächsten Generation nicht repräsentiert werden soll, ein Viertel, wenn im Durchschnitt zwei Kinder pro Ehe geboren werden und das geschlechtsreife Alter erreichen. Die betreffenden Heterozygoten können ausserdem aus irgend einem Anlass steril sein oder keine Ehe schliessen. (Eine niedrige ausserhehliche Fruchtbarkeit muss aber in Berechnung gezogen werden.) In Wirklichkeit besteht für die Anlage beim Übergang in jede neue Generation die Gefahr zufallsbedingt zu verschwinden, sie kann aber auch zufallsbedingt eine Frequenzerhöhung erfahren. In kleinen Populationen ist auch die Möglichkeit vorhanden, dass die Frequenz der Anlage in einem solchen Ausmasse zunimmt, dass man für die Anlage eine Homozygotie bekommt, was bedeutet, dass das allele Gen zufallsbedingt verschwindet. Prozesse dieser Art dürften für Tiere und Pflanzen eine wichtige Rolle spielen. Der erste, der auf diese Möglichkeit hingewiesen hat, ist HAGEDOORN (1921). Später haben SEWALL WRIGHT (1921, 1930, 1931, 1932, 1934 und 1935) und R. A. FISHER (1928, 1930 und 1931) das Problem aus verschiedenen Gesichtspunkten mathematisch analysiert. DOBZHANSKY (1937) hat auf Grund empirischer Untersuchungen das Problem besprochen und die Literatur auf diesem Gebiete referiert.

Für den Menschen dürfte man kaum Anlass haben, damit zu rechnen, dass die Mutationen zufallsbedingt innerhalb der Isolate in zivilisierten Populationen während historischer Zeit zunehmen werden, so dass eine Homozygotie entsteht. Hingegen spielt sicher die entgegengesetzte Möglichkeit, nämlich dass Mutationen zufallsbedingt verschwinden, eine grosse Rolle, weswegen man damit zu rechnen hat, dass bestimmte Anlagen in bestimmten Isolaten völlig fehlen und in anderen eine grössere oder kleinere Frequenz haben, d. h. dass die Situation entsteht, die Verfasser früher für Anlagen zu bestimmten seltenen Defekten angenommen hat. Es dürfte indessen kaum Interesse besitzen, in diesem Zusammenhange die mathematischen Untersuchungen zu referieren, die über dieses Problem ausgeführt wurden. Der hierfür Interessierte sei auf die zitierte Literatur und vor allem auf die Arbeit von DOBZHANSKY verwiesen.

## Isolat und Verwandtschaftsehe.

Als die Wirkung von Verwandtschaftsehen berechnet wurde, wurde darauf keine Rücksicht genommen, dass die Bevölkerung in Isolate aufgeteilt ist. Die Isolatgrenzen bedeuten aber, dass eine Anlage nicht beliebig selten sein kann. Damit eine Anlage in einem Isolat überhaupt existieren kann, ist wenigstens ein Heterozygot pro Generation erforderlich und dafür, dass ein Eigenschaftsträger in einem Isolat entstehen können soll, sind wenigstens 2 Heterozygoten pro Generation erforderlich. Ferner hat man damit zu rechnen, dass die Frequenz an Verwandtschaftsehen von der Isolatgrösse abhängig ist. In kleinen Isolaten kommen Verwandtschaftsehen in verhältnismässig grossem Ausmasse vor. In grossen Isolaten ist die Frequenz an Verwandtschaftsehen niedrig, was auf die grössere Möglichkeit, sich mit anderen als Verwandten zu verheiraten, zurückzuführen ist. Um eine Vorstellung darüber zu erhalten, was für eine Rolle die Verwandtschaftsehen in Populationen spielen, die aus Isolaten aufgebaut sind, können wir früher angewandte Formeln benutzen. Der Anteil an Eigenschaftsträgern  $k$ , der von Eltern abstammt, die Vetter und Base sind, ist nach Formel 45:

$$k = \frac{c(1 + 15r)}{16r}$$

Bei Panmixie ist ferner nach Formel 60 die Frequenz der Vettern-ehen  $c$ , wenn die durchschnittliche Kinderanzahl  $b$  ist (Formel 60):

$$c = \frac{2b(b-1)}{n-1}$$

Damit ein Eigenschaftsträger entstehen kann, sind wenigstens zwei Heterozygoten in einem Isolat erforderlich. In dieser Situation ist die Anlagefrequenz  $r$ , wenn die Anzahl der Individuen im Isolat  $n$  ist:

$$r = \frac{1}{n}$$

Werden diese Werte in die Formeln eingesetzt, so ergibt sich:

$$k = \frac{b(b-1)(n+15)}{8(n-1)} \quad (85)$$

Ist  $b = 2$ , was bedeutet, dass die Bevölkerung konstant ist, erhält die Formel folgendes Aussehen:

$$k = \frac{n+15}{4(n-1)}$$

und wenn  $n-1 : n \approx 1$  ist:

$$k \approx \frac{1}{4} \left( 1 + \frac{15}{n} \right)$$

Nehmen wir an, dass in jedem Isolat, in welchem die Eigenschaftsträger entstehen, im Durchschnitt 4 Heterozygoten vorhanden sind, so wird

$$r = \frac{2}{n}$$

Wird dieser Wert in die Formel eingesetzt, erhält sie folgendes Aussehen:

$$k = \frac{n+30}{8(n-1)} \text{ und } k \approx \frac{1}{8} \left( 1 + \frac{30}{n} \right) \quad (86)$$

TABELLE 22.

Prozentueller Anteil Eigenschaftsträger, die aus Vetternehen herkommen bei zufallsbedingtem Vorkommen von Vetternehen in einem Isolat von variierender Grösse ( $n$ ), wenn man annimmt, dass die Anzahl der Kinder 2 ist und dass es in jedem Isolate 2 bzw. 4 Heterozygoten gibt. Vgl. Formel 85.

Isolatgrösse = $n$	2 Heterozygoten per Isolat	4 Heterozygoten per Isolat
10	0.6945	0.5556
25	0.4167	0.2865
50	0.3316	0.2041
100	0.2875	0.1625
250	0.2650	0.1400
500	0.2575	0.1325
1 000	0.2538	0.1288
5 000	0.2508	0.1258
10 000	0.2504	0.1254
$\infty$	0.2500	0.1250



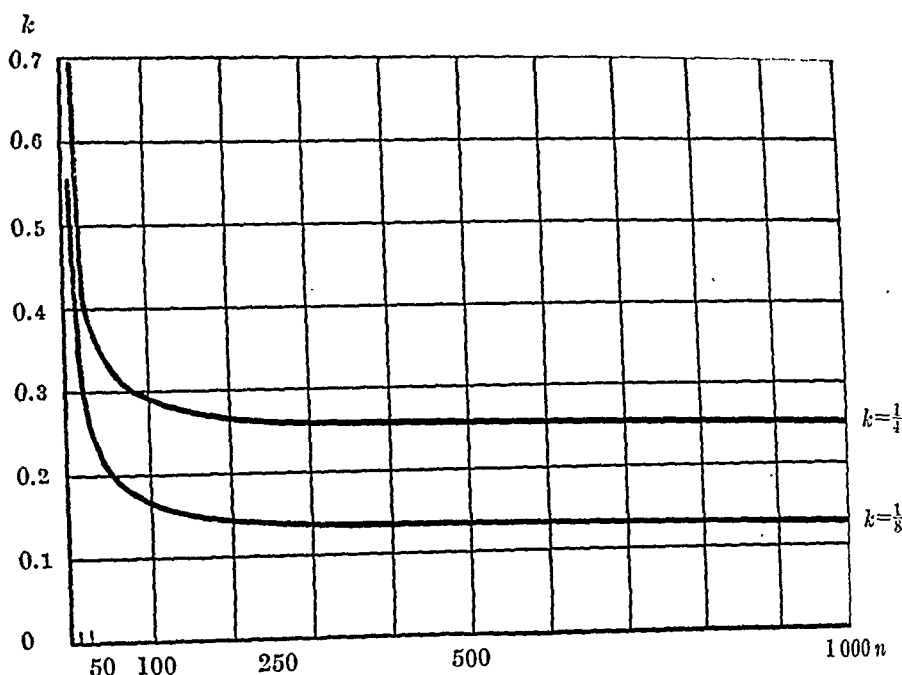


Abb. 24. Prozentueller Anteil Eigenschaftsträger, die aus Vetternehen herkommen bei zufallsbedingtem Vorkommen von Vetternehen in einem Isolat von variierender Grösse ( $n$ ), wenn man annimmt, dass die Anzahl der Kinder 2 ist und dass es in jedem Isolate 2 bzw. 4 Heterozygoten gibt. Vgl. Formel 85.

Auf Grund der beiden angegebenen Formeln wurden die Prozentwerte der Personen, die aus Vetternehen bei verschiedener Isolatgrösse stammen in Tabelle 22 und Abb. 24 angegeben. Der Grenzwert, der erhalten wird, wenn  $n = \infty$  ist, ist in einem Fall 25%, im anderen Falle 12.5%. Der Wert hält sich praktisch genommen konstant, wenn die Isolatgrösse abnimmt. Erst in sehr kleinen Isolaten, die weniger als 100 Individuen umfassen, entstehen beachtenswerte Verschiebungen. Ist  $n = 25$ , stammen im ersten Fall 42% aus Vetternehen und im anderen Falle 29%.

In Wirklichkeit dürfte man deswegen am ehesten Anlass haben, Werte zwischen 15% und 30% zu erwarten. Dies stimmt mit den Werten überein, die man bei den Untersuchungen einer Reihe seltener Eigenschaften erhält. Als Beispiel soll erwähnt werden, dass bei juveniler amaurotischer Idiotie 15% der Eigenschaftsträger aus Vetternehen stammen, bei Retinitis pigmentosa 17%, Albinismus 17%, bei Friedreich Ataxie 9% u. s. w. Die empirischen Werte scheinen mit anderen Worten etwas niedriger zu liegen, als nach der ersten Formel zu erwarten war, was darauf hindeutet, dass im Durch-



sache, ob man in erster Linie an Verwandtschaftsehen denken will und untersucht, welche Bedeutung die Verminderung an Verwandtschaftsehen in weiterem Sinne besitzt. Nachdem man nur Angaben über Verwandtschaftsehen näheren Grades (Vetternehen) erhalten kann, ist man gezwungen von allen schwächeren Graden einer Verwandtschaftsehe abzusehen. Wir haben bereits früher hervorgehoben, dass prinzipiell gesehen alle Ehen Verwandtschaftsehen sind; denn eine Berechnung der Anzahl von Personen, die in früheren Generationen vorhanden sein müssen, wenn niemals zwischen ihnen Verwandtschaftsehen vorgekommen wären, führt schnell zu astronomischen Werten, während in Wirklichkeit frühere Generationen sicher kleiner waren als die nun lebenden.

Geht man hingegen davon aus, dass Isolatgrenzen vorhanden waren und aufgehoben worden sind, ferner dass Verwandtschaftsehen in dem Ausmasse vorgekommen sind, wie sie zufallsbedingt im Hinblick auf die vorhandenen Isolatgrenzen zu erwarten waren, so hält man sich an das, was faktisch geschehen ist. Man schematisiert indessen die Situation dadurch, dass man Isolate mit scharfen Grenzen annimmt, und zwar auf dieselbe Weise, wie man die Situation schematisiert, wenn man über scharf abgegrenzte Generationen spricht, während in Wirklichkeit die Generationen unbemerkt ineinander übergehen. Eine Schematisierung dieser Art dürfte indessen in den meisten Fällen nicht zu irreführenden Ergebnissen führen.

Ein Spezialfall, der theoretisches Interesse besitzt, ist ein scharf abgegrenztes Isolat (z. B. die Bevölkerung einer Insel), das auf Grund einer hohen Nativität an Grösse zunimmt. In einem solchen Isolat würde man erwarten, dass die Frequenz an Vetternehen abnimmt, während hingegen die Frequenz an Ehen zwischen entfernter Verwandten zunimmt. Dies balanciert so, dass die Anlagefrequenz und die Frequenz der Eigenschaftsträger unverändert bleibt. Betrachtet man die Population als ein Isolat, so gelangt man ohne weiteres zu dem Ergebnis, dass die Frequenz der Eigenschaftsträger konstant ist, während man hingegen bei der Untersuchung der Frequenz der Vetternehen leicht zu dem Schluss verleitet wird, dass die Frequenz an Eigenschaftsträgern abgenommen hat, und zwar deswegen, weil man schwächere Grade an Verwandtschaftsehen nicht berücksichtigt hat. Wie HALDANE hervorgehoben hat, können beide Betrachtungsweisen in verschiedenen Situationen spezielle Vorteile bedeuten. Es kann deswegen angebracht sein, bei der Lösung von



machen, weisen verschiedene Arten von Defekten auf, von denen jeder für sich sehr selten vorkommt. Dasselbe gilt wahrscheinlich auch für Idiotie und Verkrüppelung, soweit es sich hier um erbliche Eigenschaften handelt. Für Taubstummheit ist die Situation noch kaum aufgeklärt, es ist aber möglich, dass diese Gruppe einheitlicher ist. Durch ein Verbot der Vetternehe dürfte man also einen deutlichen Effekt auf die Summe der Defekte erhalten. Diese Wirkung wird nach einer Generation erreicht und dann gewinnt man nichts mehr durch weitere Massnahmen. Erlaubt man erneut Verwandtschaftsehen, kommt die erhöhte Frequenz zurück. Gegenüber dem Gewinn, der erhalten werden kann, muss aber der Verlust abgewogen werden, den man evt. dadurch erhält, dass seltene Eigenschaften vorteilhafter Art auch eine verminderte Frequenz erhalten. In der Masse wie eine spezielle Begabung durch seltene monohybride rezessive Anlagen bedingt wird, kann man eine Abnahme auch derselben erwarten. Es ist indessen nicht wahrscheinlich, dass eine besondere Begabung auf diese einfache Art vererbt wird. (Es ist selbstverständlich auch vorstellbar, dass bestimmte Defekte eine kompliziertere Vererbung haben, obwohl die Mehrzahl einen einfachen Erbmechanismus aufzuweisen scheint.) Für Eigenschaften mit polyhybridem Charakter ist die Wirkung geringer als für monohybride Eigenschaften. Unser Wissen darüber, wie Eigenschaften vererbt werden, ist gegenwärtig mangelhaft, und ehe man definitiv vom Vererbungsstandpunkt aus zu diesen Fragen Stellung nimmt, dürften spezielle Untersuchungen erforderlich sein. In diesem Zusammenhange wollten wir nur gewisse Gesichtspunkte andeuten, die wichtig sind. Der Vollständigkeit halber soll vielleicht auch betont werden, dass soziale Gesichtspunkte selbstverständlich auch beachtet werden müssen. Man kann es nicht direkt bewerten, was es für einzelne Personen bedeutet, wenn deren Handlungsfreiheit dadurch eingeschränkt wird, dass man Vetternehen verbietet; diese Seite des Problems ist selbstverständlich nicht gleichgültig.



handen, die aus Vererbungsgesichtspunkten unterschiedlich beschaffen sind. Man kann auch hier von Gattenwahl sprechen, die mit Hilfe von Isolatgrenzen vorsieht. Im Gegensatz zu dem aus Personen mit Defekten bestehenden Isolat werden ja diese Isolate nicht für jede Generation neu gebildet, sondern besitzen einen permanenteren Charakter.

### Gattenwahl durch Isolate, die aus Personen mit Defekten bestehen.

Wir nehmen an, dass durch Gattenwahl in Isolaten, die aus Personen mit Defekten bestehen, die Frequenz der Eigenschaftsträger für eine monohybride rezessive Eigenschaft nach  $n$  Generationen  $r^2 + \Delta_n$  ist. Für die Heterozygoten ist dann die Frequenz  $2rd - 2\Delta_n$  und für die dominanten Homozygoten ist die Frequenz  $d^2 + \Delta_n$ . Wir nehmen ferner an, dass ein Anteil  $k_1$  der rezessiven Eigenschaftsträger in dem aus Personen mit Defekten bestehenden Isolat enthalten ist und dass von den Heterozygoten und den dominanten Homozygoten  $k_2$  in diesem Isolat enthalten sind. Wir erhalten dann im Isolat die Zusammensetzung an Ehen, die in Tabelle 23 angegeben wird, in welcher auch die Beschaffenheit der Nachkommen angeführt ist.

Die Nachkommen im Isolat werden Defekte in folgender Frequenz aufweisen wobei man Rücksicht auf die Isolatgrösse im Vergleich zu der Population nehmen muss.

$$r_a^2(n+1) = \frac{[k_2 r + (k_1 - k_2) r_n^2]^2}{k_2 + (k_1 - k_2) r_n^2} \quad (87)$$

In den übrigen Populationen wird der Charakter der Ehen und der Nachkommen in Tabelle 24 angegeben.

Die Nachkommen werden also die rezessive Eigenschaft mit folgender Frequenz aufweisen:

$$r_b^2(n+1) = \frac{[(1 - k_2) r - (k_1 - k_2) r_n^2]^2}{(1 - k_2) - (k_1 - k_2) r_n^2} \quad (88)$$

Wir erhalten also für die ganze Population folgende Rekursionsformel:

$$r_{n+1}^2 = \frac{[k_2 r + (k_1 - k_2) r_n^2]^2}{k_2 + (k_1 - k_2) r_n^2} + \frac{[(1 - k_2) r - (k_1 - k_2) r_n^2]^2}{(1 - k_2) - (k_1 - k_2) r_n^2} \quad (89)$$

TABELLE 23.

Vgl. Text.

Elternkombinationen	Häufigkeit in der Defektsisolat	Kinder		
		Häufigkeit dem Typen		
		$RR_n$	$RD_n$	$DD_n$
$RR_{n-1} \times RR_{n-1}$	$k_1^2 (r^2 + d)^2 = a$	$a$		
$RR_{n-1} \times RD_{n-1}$	$2 k_1 k_2 (r^2 + d) (2 rd - 2 d) = b$	$\frac{1}{2} b$	$\frac{1}{2} b$	
$RR_{n-1} \times DD_{n-1}$	$2 k_1 k_2 (r^2 + d) (d^2 + d) = c$		$c$	
$RD_{n-1} \times RD_{n-1}$	$k_2^2 (2 rd - 2 d)^2 = d'$	$\frac{1}{4} d'$	$\frac{1}{2} d'$	$\frac{1}{4} d'$
$RD_{n-1} \times DD_{n-1}$	$2 k_1 k_2 (2 rd - 2 d) (d^2 + d) = e$		$\frac{1}{2} e$	$\frac{1}{2} e$
$DD_{n-1} \times DD_{n-1}$	$k_2^2 (d^2 + d)^2 = f$			$f$
Summen		$RR_n = a + \frac{1}{2} b + \frac{1}{4} d'$	$RD_n = \frac{1}{2} b + c + \frac{1}{2} d' + \frac{1}{2} e$	$DD_n = \frac{1}{4} d' + \frac{1}{2} e + f$



TABELLE 24.

Vgl. Text.

Elternkombina- tionen	Häufigkeit in der übrige Population	Kinder		
		Häufigkeit der Typen		
		RR <sub>n</sub>	RD <sub>n</sub>	DD <sub>n</sub>
RR <sub>n-1</sub> × RR <sub>n-1</sub>	$(1 - k_1)^2 (r^2 + d)^2 = a$	a	$\frac{1}{2} b$	
RR <sub>n-1</sub> × RD <sub>n-1</sub>	$2(1 - k_1)(1 - k_2)(r^2 + d)(2rd - 2d) = b$	$\frac{1}{2} b$	c	
RR <sub>n-1</sub> × DD <sub>n-1</sub>	$2(1 - k_1)(1 - k_2)(r^2 + d)(d^2 + d) = c$	$\frac{1}{4} d'$	$\frac{1}{2} d'$	$\frac{1}{4} d'$
RD <sub>n-1</sub> × RD <sub>n-1</sub>	$(1 - k_2)^2 (2rd - 2d)^2 = d'$		$\frac{1}{2} e$	$\frac{1}{2} e$
RD <sub>n-1</sub> × DD <sub>n-1</sub>	$2(1 - k_2)^2 (2rd - 2d)(d^2 + d) = e$			f
DD <sub>n-1</sub> × DD <sub>n-1</sub>	$(1 - k_2)^2 (d^2 + d)^2 = f$			
Summen		RR <sub>n</sub> $a + \frac{1}{2} b + \frac{1}{4} d'$	RD <sub>n</sub> $\frac{1}{2} b + c + \frac{1}{2} d' + \frac{1}{2} e$	DD <sub>n</sub> $\frac{1}{4} d' + \frac{1}{2} e + f$



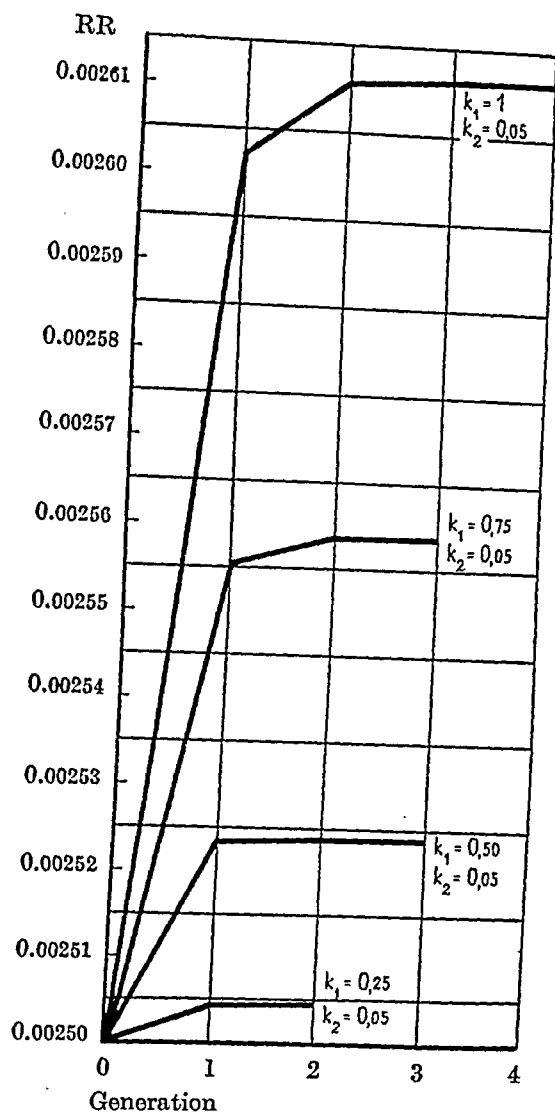


Abb. 25.

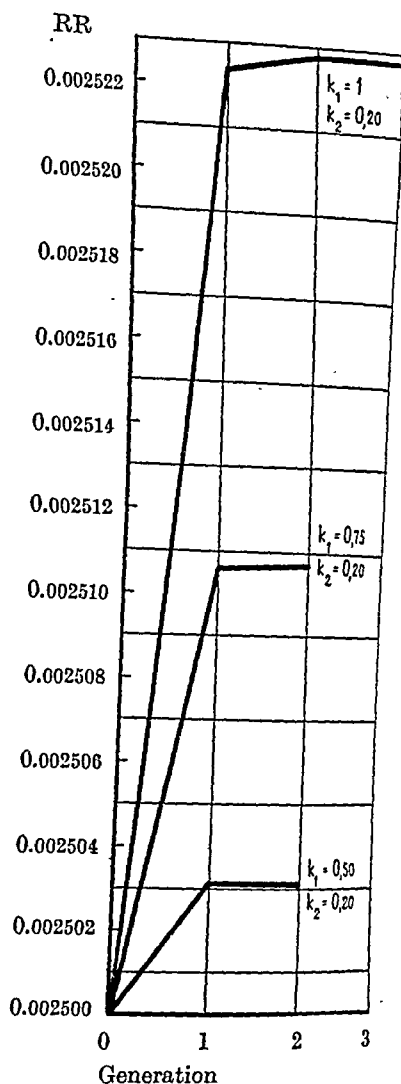


Abb. 26.

Abb. 25 und 26. Häufigkeit von Eigenschaftsträgern in Totalpopulationen bei Vorkommen von Gattenwahl in Defektisolaten bei variierenden Werten von  $k_1$  und  $k_2$  nach verschiedenen Generationen:  $RR_0$ ,  $RR_1$ ,  $RR_2$ ,  $RR_3$  und  $RR_4$ . Die Rechenoperation wurde, praktisch genommen, bis zur Gleichgewichtslage durchgeführt. Vgl. Formel 89.

die Personen mit Defekten eine besonders geringe Fruchtbarkeit haben, so bedeutet ja dies eine Selektion. Durch diese nimmt die Frequenz der erblich bedingten Eigenschaftsträger, die den Defekt haben, sowie auch die Heterozygotenfrequenz ab. Eine Selektion, die in Zusammenhang mit Gattenwahl durch Isolatneubildung wirkt, hat im Prinzip denselben Effekt wie Selektion bei Panmixie. Wenn die Eigenschaftsträger beginnen selten zu werden, werden sie praktisch

genommen immer von Eltern abstammen, die Heterozygoten sind. Wir erreichen das Gleichgewicht zwischen Selektion und Mutationsfrequenz, und die Frequenz der Heterozygoten in dieser Gleichgewichtslage wird unbedeutend niedriger sein als in derselben Gleichgewichtslage bei Panmixie. Gattenwahl beschleunigt etwas die Selektion auf dem Wege zu dieser Gleichgewichtslage, handelt es sich aber um seltene Eigenschaften, so ist der Unterschied unwesentlich.

Ein Prozess dieser Art, wie der hier angegebene, dürfte in gewissem Ausmasse möglicherweise nicht nur für Eigenschaften eine Rolle spielen, die den Charakter eines Defektes aufweisen, sondern auch für solche, die mit einer besonderen Einstellung der Interessen zusammenhängen, in dem Masse wie solche erblich bedingt sind. Man kann z. B. erwarten, dass musikalische Personen auf Grund ihrer Interessen sich in besonderem Ausmasse in bestimmten Umgangs-kreisen und Organisationen treffen werden. Man dürfte indessen auch damit rechnen müssen, dass auch in Städten, in welchen mehr oder minder ausgeprägte musikalische Isolate vorhanden sind, gleichzeitig in gewissem Ausmasse auch eine Gattenwahl ausserhalb dieser Isolate zwischen musikalischen Personen vorkommt. Gegenwärtig sind aber keine Angaben zugänglich, die Anhaltspunkte für die Beurteilung der Rolle von Interesseisolaten und von durch Interessen bedingter Gattenwahl geben würden; es ist ferner kaum untersucht, in welchem Masse spezielle Interessen erblich bedingt sind.

### Gattenwahl und soziale Isolate.

Es ist eine allgemein verbreitete Vorstellung, dass ein Unterschied in der erblichen Beschaffenheit zwischen verschiedenen Gesellschaftsklassen vorhanden ist. Die obere Schicht soll eine Auswahl bestimmter vorteilhafter Eigenschaften darstellen. Im Hinblick auf diese Vorstellung ist es von Interesse einen Versuch anzustellen, um zu ermitteln, welche Wirkung die Gattenwahl über die Isolatgrenzen oder Selektion bei Bildung von Isolaten bedingt. Es ist auch hier eine Geschmacksache, ob man den Nachdruck auf die Selektion legen will oder ob man den Prozess als eine Art Gattenwahl von besonderem Charakter ansehen will.

Wir gehen von der Annahme aus, dass ursprünglich eine Panmixie vorgelegen hat und dass die Bevölkerung in zwei Klassen aufgeteilt

wurde, von welchen die eine als verhältnismässig klein angesehen werden kann, beide haben aber ursprünglich dieselbe Anlagebeschaffenheit gehabt. Wir nehmen ferner an, dass eine bestimmte Zirkulation zwischen den Ständen und zwischen den beiden Isolaten stattgefunden hat, so dass Personen mit einer bestimmten monohybriden Eigenschaft aus dem grösseren Isolat eine gewisse Möglichkeit besitzen, dem kleineren Isolat anzugehören. Wir sehen dann von der Möglichkeit ab, dass Personen zufällig in das kleinere Isolat gelangen und dass Personen, denen die betreffende Eigenschaft fehlt, zufallsbedingt in das grössere Isolat versetzt werden. Wir nehmen mit anderen Worten an, dass diese beiden Verschiebungen einander aufheben. Würde dies indessen nicht der Fall sein, so spielt es keinerlei prinzipielle Rolle für die Natur des Prozesses, den wir untersuchen wollen. Die Überführung bestimmter Eigenschaftsträger, die angenommen wurde, bedeutet, dass das kleinere Isolat auf Kosten des grösseren wächst.

Das eine Isolat ist also ausschliesslich einer negativen Selektion ausgesetzt und wird Eigenschaftsträger eines bestimmten Typus beraubt. (Dieses Isolat entspricht also der Unterklasse, welche Verluste an begabten Personen, die sich heraufarbeiten, erleidet.) Wir können dann die früheren Formeln für Selektion anwenden (Formel 29). Hat also die rezessive Eigenschaft die Frequenz  $r$  und werden  $k$  der Eigenschaftsträger aus einem Isolat weggenommen, so gilt bei monohybrider Rezessivität:

$$r_{n+1}^2 = \frac{r_n^2 (1 - kr_n)^2}{(1 - kr_n^2)^2}$$

Betrifft die Selektion die dominante Eigenschaft (Vgl. Formel 32), so wird die Anlagefrequenz und die Frequenz an Eigenschaften kontinuierlich abnehmen. Insoweit die betreffende Eigenschaft selten ist, hat man ja in der Praxis damit zu rechnen, dass die Eigenschaft praktisch genommen immer in heterozygoter Form vorkommt und die Verschiebung wird dann ziemlich proportional mit der Stärke der Auswahl, die vorliegt, verlaufen. Wird z. B.  $\frac{1}{10}$  der Eigenschaftsträger pro Generation in das kleinere Isolat überführt, so wird die Eigenschaft praktisch genommen in dem grossen Isolat in etwa 10 Generationen ausgerottet sein. Dieser Fall besitzt aber kaum Interesse, da man sich kaum vorstellen kann, dass die Eigenschaften, um die es sich hier handelt, auf diese Weise vererbt werden. Es muss sich um

TABELLE 26.

Vgl. Text.

Elternkombinationen	Häufigkeit in der kleine Isolat	Kinder			
		Häufigkeit der Typen			
		RR <sub>n</sub>	RD <sub>n</sub>	DD <sub>n</sub>	
RR <sub>n-1</sub> × RR <sub>n-1</sub>	$(A_n x_n^2 + B_n k r_n^2)^2 = a$	a			
RR <sub>n-1</sub> × RD <sub>n-1</sub>	$2(A_n x_n^2 + B_n k r_n^2) 2 A_n x_n y_n = b$	$\frac{1}{2} b$	$\frac{1}{2} b$		
RR <sub>n-1</sub> × DD <sub>n-1</sub>	$2(A_n x_n^2 + B_n k r_n^2) A_n y_n^2 = c$		c		
RD <sub>n-1</sub> × RD <sub>n-1</sub>	$4 A_n^2 x_n^2 y_n^2 = d'$	$\frac{1}{4} d'$	$\frac{1}{2} d'$	$\frac{1}{4} d'$	
RD <sub>n-1</sub> × DD <sub>n-1</sub>	$4 A_n^2 x_n y_n^3 = e$		$\frac{1}{2} e$	$\frac{1}{2} e$	
DD <sub>n-1</sub> × DD <sub>n-1</sub>	$A_n^2 y_n^4 = f$			f	
	Summen	RR <sub>n</sub> $a + \frac{1}{2} b + \frac{1}{4} d'$	RD <sub>n</sub> $\frac{1}{2} b + c + \frac{1}{2} d' + \frac{1}{2} e$	DD <sub>n</sub> $\frac{1}{4} d' + \frac{1}{2} e + f$	

Eigenschaften handeln die auf kompliziertere Art vererbt werden. Es ist deswegen interessanter die Wirkung der Selektion auf eine rezessive Eigenschaft zu untersuchen. Eigenschaften, die kompliziert vererbt werden, z. B. dihybride dominante Eigenschaften u. s. w., verhalten sich auf ähnliche Weise wie monohybride rezessive Eigenschaften.

Wir nehmen ein kleines Isolat  $B$  an, das aus einem anderen Isolat  $A$  einen Zuschuss an rezessiven Eigenschaftsträgern erhält, wobei  $B + A = 1$ . Wir nehmen ferner an, dass die rezessive Anlage in  $B$  die Frequenz  $r$  hat und dass  $A$  die rezessive Anlage mit der Frequenz  $x$  und die Dominante mit der Frequenz  $y$  aufweist. Es ist also  $x + y = 1$ . Wir nehmen wie vorher an, dass  $k$  der Eigenschaftsträger in  $B$  nach  $A$  überführt werden. Die Zusammensetzung der Ehen und Nachkommen in dem kleineren Isolat ergibt sich aus Tabelle 26.

Wenn man Rücksicht nimmt auf die Veränderungen der relativen Isolatgrösse erhält man die folgende Rekursionsformel:

$$x_{n+1}^2 = \left[ \frac{A_n x_n + B_n k r_n^2}{A_n + B_n k r_n^2} \right]^2 \quad (90)$$

wobei  $r_n$  aus der oben angegebenen Formel 29 erhalten wird und

$$A_{n+1} = A_n + B_n k r_n^2$$

sowie

$$B_{n+1} = B_n (1 - k r_n^2)$$

Die gesamte Frequenz der Eigenschaftsträger in der gesamten Population  $p_n$  wird

$$p_n = B_n r_n^2 + A_n x_n^2 \quad (91)$$

Mit Hilfe der oben angegebenen Formeln wurden die Werte in Tabelle 27, 28 und 29 berechnet. Wir haben uns damit begnügt von einer Eigenschaft auszugehen, die so selten ist, dass sie bei Panmixie bei einer Person auf 1000 vorkommen würde und haben ferner angenommen, dass das kleinere Isolat 5 % der Population ausmacht und das grössere also 95 % umfasst. Vgl. Abb. 27 und 28.

Betrachten wir zuerst die Unterklasse, so finden wir, dass die Verschiebung bei schwacher Selektion einen sehr geringen Effekt hat. Geht 1 % der Eigenschaftsträger pro Generation in das Oberklassenisolat über, so nimmt deren Frequenz in der Unterklasse von 0.1 % auf 0.0994 % in 10 Generationen ab. Rechnet man für eine

TABELLE 27.

Beschaffenheit der Unterklasse in aufeinanderfolgenden Generationen, wenn diese 95 % der Population ausmacht, die Eigenschaftsträger die Frequenz 1 % haben und die Selektion zur Oberklasse die Frequenz 1 % ( $k = 0.01$ ) bis 100 % ( $k = 1$ ) hat, nach der Formel 29. Die angegebenen Zahlen gelten auch für partielle Selektion von variierender Stärke für eine rezessive, monohybride Eigenschaft in der Bevölkerung.

Gen.	$k = 0.01$	$k = 0.05$	$k = 0.10$	$k = 0.25$	$k = 0.50$	$k = 1$
0	0.0010000	0.0010000	0.0010000	0.0010000	0.0010000	0.0010000
1	0.0009994	0.0009969	0.0009939	0.0009847	0.0009606	0.0009396
2	0.0009988	0.0009939	0.0009878	0.0009698	0.0009406	0.0008846
3	0.0009982	0.0009909	0.0009818	0.0009552	0.0009129	0.0008329
4	0.0009976	0.0009879	0.0009759	0.0009410	0.0008864	0.0007868
5	0.0009970	0.0009849	0.0009700	0.0009271	0.0008612	0.0007445
6	0.0009964	0.0009819	0.0009642	0.0009135	0.0008368	0.0007055
7	0.0009958	0.0009789	0.0009584	0.0009002	0.0008134	0.0006695
8	0.0009952	0.0009759	0.0009527	0.0008871	0.0007910	0.0006362
9	0.0009946	0.0009730	0.0009470	0.0008743	0.0007695	0.0006053
10	0.0009940	0.0009701	0.0009414	0.0008618	0.0007489	0.0005766
15	0.0009910	0.0009556	0.0009140	0.0008031	0.0006572	0.0004596
20	0.0009880	0.0009415	0.0008878	0.0007503	0.0005812	0.0003749

TABELLE 28.

Beschaffenheit der Oberklasse in aufeinanderfolgenden Generationen, wenn diese 5 % der Population ausmacht und die Eigenschaftsträger die Frequenz 1 % haben, ferner die Selektion zur Oberklasse die Frequenz 1 % ( $k = 0.01$ ) bis 100 % ( $k = 1$ ) hat, nach der Formel 90.

Gen. $x^2$	$k = 0.01$	$k = 0.05$	$k = 0.10$	$k = 0.25$	$k = 0.50$	$k = 1$
0	0.001000	0.001000	0.001000	0.001000	0.00100	0.00100
1	0.001011	0.001059	0.001119	0.001309	0.00165	0.00243
2	0.001023	0.001110	0.001244	0.001649	0.00242	0.00428
3	0.001035	0.001181	0.001373	0.002020	0.00330	0.00640
4	0.001046	0.001244	0.001508	0.002419	0.00425	0.00872
5	0.001058	0.001308	0.001649	0.002842	0.00528	0.01117
6	0.001070	0.001374	0.001793	0.003288	0.00637	0.01368
7	0.001081	0.001441	0.001943	0.003755	0.00751	0.01624
8	0.001093	0.001509	0.002097	0.004242	0.00868	0.01851
9	0.001105	0.001579	0.002255	0.004746	0.00989	0.02136
10	0.001117	0.001649	0.002417	0.005267	0.01112	0.02389
15	0.001178	0.002019	0.003284	0.008064	0.01747	0.03582
20	0.001241	0.002417	0.004238	0.011088	0.02384	0.04639



TABELLE 29.

Beschaffenheit der Totalpopulation, wenn diese in eine Oberklasse, die 5 % der Bevölkerung und eine Unterklasse, die 95 % der Bevölkerung umfasst, eingeteilt ist, und wenn die Eigenschaftsträger die Frequenz 1 %<sub>00</sub> haben bei verschiedener Stärke einer selektiven Verschiebung von Unterklasse zu Oberklasse von verschiedener Intensität ( $k = 0.01$  bis  $k = 1$ ). Vgl. Formel 91.

Gen.	$k = 0.01$	$k = 0.05$	$k = 0.10$	$k = 0.25$	$k = 0.50$	$k = 1$
0	0.0010000	0.0010000	0.0010000	0.001000	0.001000	0.001000
1	0.0010000	0.0010000	0.0010002	0.001001	0.001004	0.001016
5	0.0010001	0.0010011	0.0010042	0.001025	0.001092	0.001309
10	0.0010002	0.0010043	0.0010166	0.001092	0.001310	0.001915
15	0.0010004	0.0010096	0.0010315	0.001190	0.001596	0.002578
20	0.0010007	0.0010167	0.0010613	0.001310	0.001915	0.003218

Generation 25 Jahre, so umfasst dieser Zeitraum 250 Jahre. Werden 10 % auf die Oberklasse überführt, so nimmt die Frequenz der Eigenschaftsträger in derselben Zeit auf 0.09414 % ab. Erst bei höheren Werten für die Selektion erhält man eine deutliche Wirkung. Wird die Hälfte der Eigenschaftsträger überführt, so wird nach 10 Generationen die Frequenz in der Unterklasse 0.07489 %. Theoretisch gesehen kann es möglicherweise von Interesse sein festzustellen, dass falls alle Eigenschaftsträger überführt werden, die Frequenz in 10 Generationen auf etwas mehr als die Hälfte, nämlich 0.05766 % sinkt.

Betrachten wir die Oberklasse, so finden wir, dass bei schwacher Selektion, wenn nur 1 % der Eigenschaftsträger sich heraufarbeitet, die Frequenz der Eigenschaft in 10 Generationen auf 0.1117 % steigt. Beträgt die Selektion 10 %, handelt es sich um eine deutlichere Verschiebung. Nach derselben Zeit beträgt dann die Frequenz 0.2417 %. Die Eigenschaftsträger sind mit anderen Worten dann in der Oberklasse etwas mehr als doppelt so viele wie in der Unterklasse. Bei extrem hohen Werten für die Selektion wird die Verschiebung sehr kräftig. Hat sich die Hälfte der Eigenschaftsträger heraufgearbeitet, so steigt die Frequenz auf 1.112 %, haben sich alle heraufgearbeitet, so erreicht sie in 10 Generationen eine Frequenz von 2.389 %. Die Zahl illustriert, dass eine selektive Ständeselektion keine oder nur eine geringe Bedeutung für die Frequenz der Eigenschaftsträger in der Unterklasse besitzt, dessen ungeachtet kann man sich vorstellen, dass sie eine nicht unwesentliche Wirkung auf die Beschaffenheit der Oberklasse ausübt.

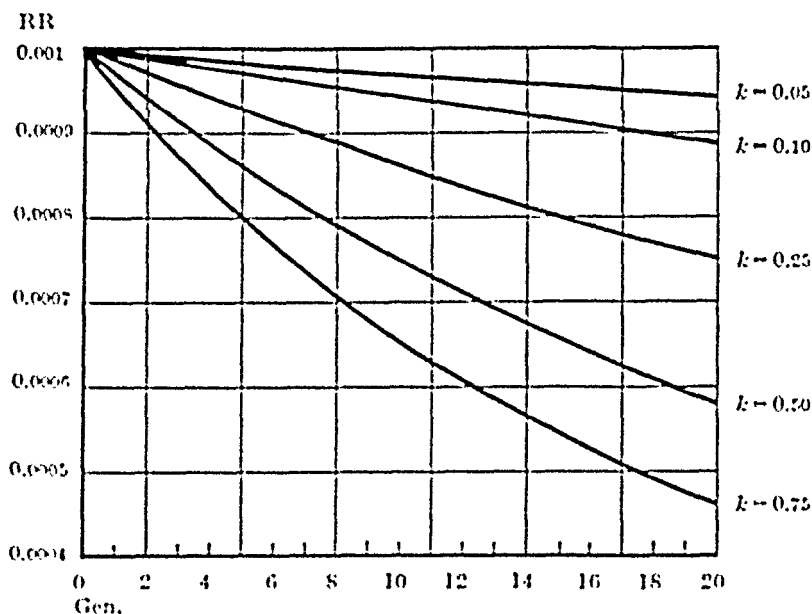


Abb. 27. Beschaffenheit der Unterklasse in aufeinanderfolgenden Generationen, wenn diese 95 % der Population ausmacht, die Eigenschaftsträger die Frequenz 1 %<sub>00</sub> haben und die Selektion zur Oberklasse die Frequenz 5 % ( $k = 0.05$ ) bis 75 % ( $k = 0.75$ ) hat, nach der Formel 29. Die angegebenen Zahlen gelten auch für partielle Selektion von variierender Stärke für eine rezessive, monohybride Eigenschaft in der Bevölkerung.

Schliesslich ist es von Interesse zu versuchen, eine Vorstellung davon zu erhalten, was eine selektive Isolatumstellung vom Gesichtspunkt der ganzen Population aus bedeutet. Offenbar muss die Wirkung die sein, dass die Frequenz der Eigenschaftsträger, verglichen mit Panmixie, gleichzeitig zunimmt wie die Heterozygoten abnehmen und je stärker die Selektion ist, eine desto grössere Bedeutung muss ja der Prozess haben. Bei schwacher Selektion wird keine nennenswerte Verschiebung erhalten. Wenn die selektive Zirkulation zwischen den Ständen 1 % der Eigenschaftsträger betrifft, so steigt deren Frequenz innerhalb von 10 Generationen von 0.1 % auf 0.10002 %. Umfasst die Selektion die Hälfte der Eigenschaftsträger, so ist die Verschiebung unbedeutend, aber doch deutlich. Die Frequenz steigt in 10 Generationen auf 0.1310 %. Bei totaler Selektion wird eine so starke Wirkung erhalten, dass die Frequenz der Eigenschaftsträger auf 0.1915 steigt, d. h. fast doppelt so hoch ist wie zu Beginn des Prozesses.

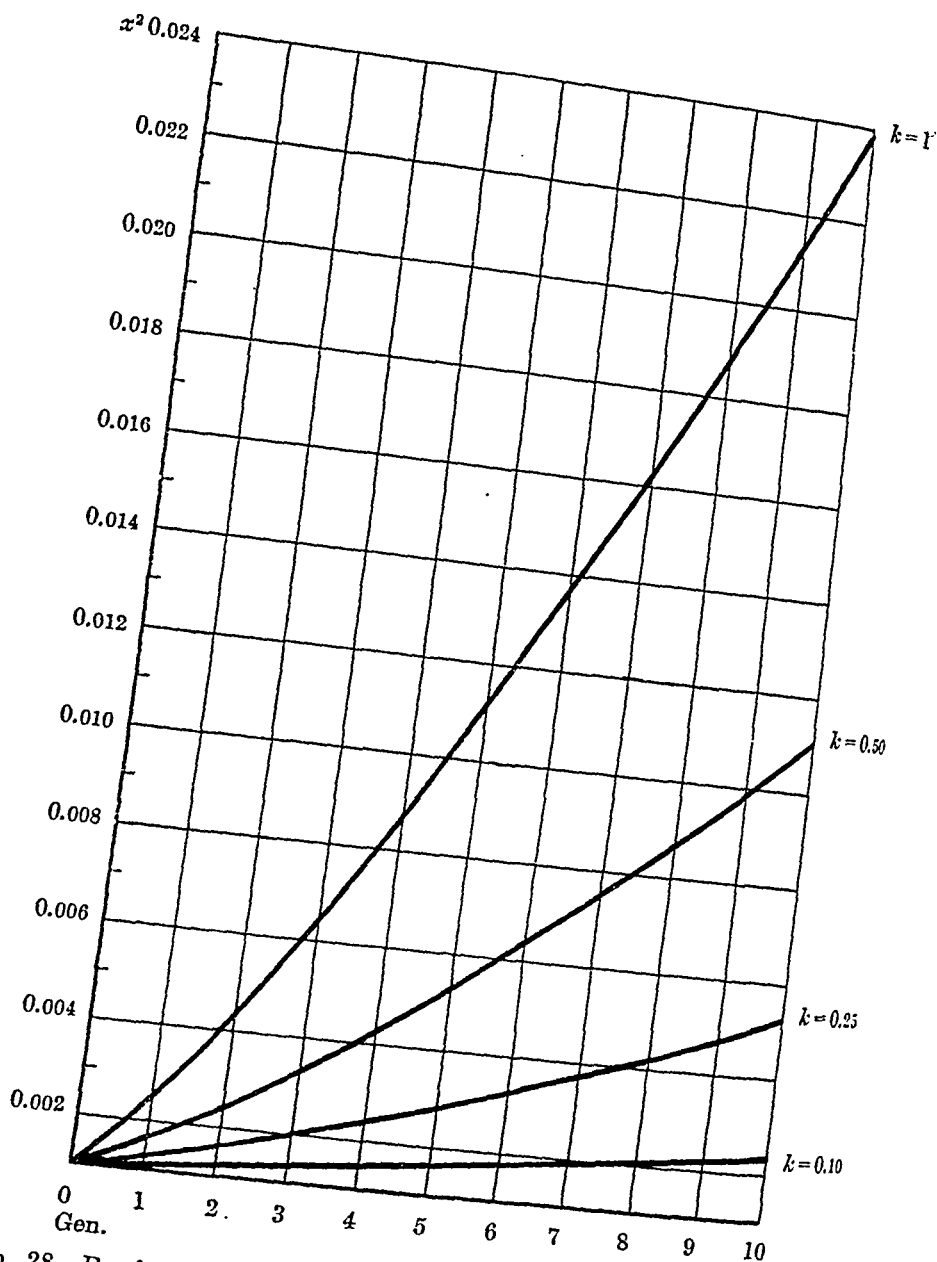


Abb. 28. Beschaffenheit der Oberklasse in aufeinanderfolgenden Generationen, wenn diese 5 % der Population ausmacht und die Eigenschaftsträger die Frequenz 1 % haben, ferner die Selektion zur Oberklasse die Frequenz 10 % ( $k = 0.1$ ) bis 100 % ( $k = 1$ ) hat, nach der Formel 90.

In Abb. 29 und 30 werden die Veränderungen gezeigt, die in der Ober- und Unterklasse vorsehgehen, wenn die erstere von Anfang an 5 % der Bevölkerung ausmacht und die Eigenschaft die Frequenz 1 % sowie die Selektion variierende Stärke hat. Es fehlen nun empirische Unterlagen, die Berechnungen ermöglichen würden, die sich auf das beziehen, was wirklich geschehen

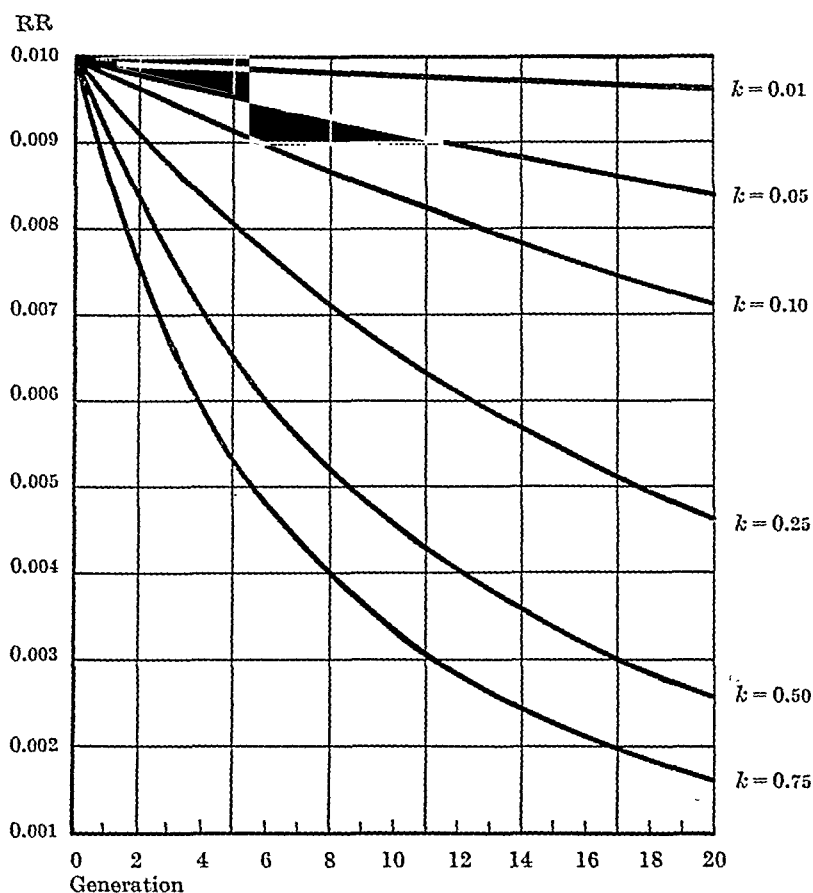


Abb. 29. Beschaffenheit der Unterklasse in aufeinanderfolgenden Generationen, wenn diese 95 % der Population ausmacht, die Eigenschaftsträger die Frequenz 1 % haben und die Selektion zur Oberklasse die Frequenz 1 % ( $k = 0.01$ ) bis 75 % ( $k = 0.75$ ) hat, nach der Formel 29. Die angegebenen Zahlen gelten auch für partielle Selektion von variierender Stärke für eine rezessive, monohybride Eigenschaft in der Bevölkerung.

ist und geschieht. Die Verhältnisse sind selbstverständlich in verschiedenen Ländern sowie unter verschiedenen zeitlichen Perioden unterschiedlich. Die Eigenschaften, die in diesem Zusammenhang interessieren, sind ferner nicht von monohybridem rezessivem Charakter, sondern werden auf eine kompliziertere Art vererbt. Man kann sich vorstellen, dass die Selektion gegen solche Eigenschaften wie Intelligenz, künstlerische Begabung, moralische Qualifikationen, Aussehen u. s. w. gerichtet ist. In welchem Ausmasse und auf welche Weise Eigenschaften dieser Art erblich bedingt sind, ist ja noch nicht untersucht worden und es ist noch weniger bekannt,

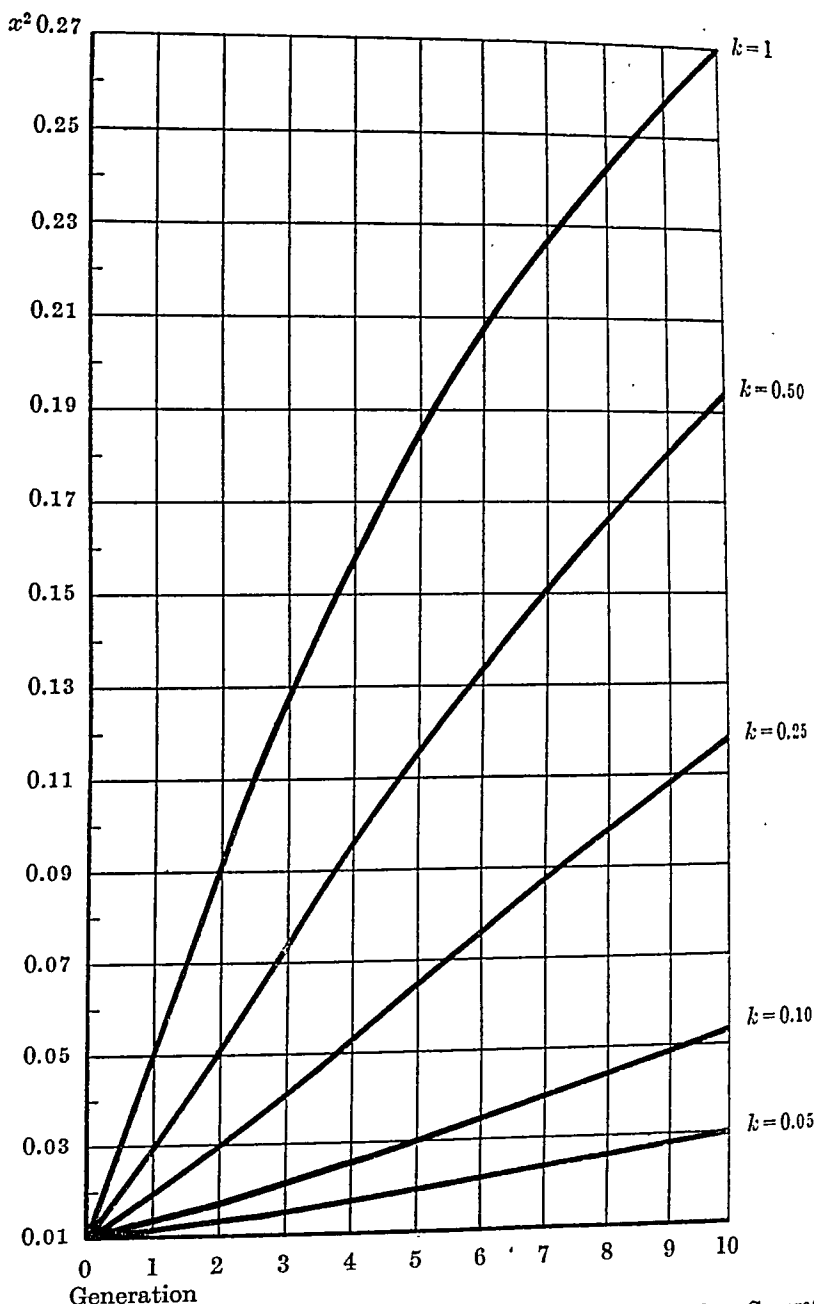


Abb. 30. Beschaffenheit der Oberklasse in aufeinanderfolgenden Generationen, wenn diese 5 % der Population ausmacht und die Eigenschaftsträger die Frequenz 1 % haben, ferner die Selektion zur Oberklasse die Frequenz 5 % ( $k = 0.05$ ) bis 100 % ( $k = 1$ ), hat, nach der Formel 90.

in welchem Ausmasse eine Zirkulation zwischen den Ständen vor sich geht und selektiven Charakter besitzt. Das Problem wurde hier absichtlich vereinfacht. Wir haben uns damit begnügt, den Prozess nur im Hinblick auf eine einfache monohybride rezessive Eigenschaft

zu analysieren und unter der Annahme, dass nur zwei Isolate vorhanden sind, ferner haben wir willkürlich angenommen, dass das kleinere Isolat 5 % der Bevölkerung umfasst. Unsere Berechnungen haben also sehr wenig Berührung mit dem, was wirklich vorsichgeht, und man ist vielleicht versucht zu behaupten, dass diese Berührung so gering ist, dass die Werte nicht einmal theoretisches Interesse besitzen.

Mit den Berechnungen wurde aber in erster Linie beabsichtigt, die Aufmerksamkeit auf das Problem zu richten und eine Andeutung davon zu geben, nach welchen Linien es gelöst werden kann. Erst wenn man sich allgemeiner für Fragen dieser Art zu interessieren beginnt, ist es möglich, dass wir allmählich Ausgangspunkte erhalten werden, die einer rationellen Besprechung dieser Frage zugrundegelegt werden können; es stösst selbstverständlich auf keine Schwierigkeit, mathematisch Ausdrücke zu entwickeln, die für Eigenschaften gelten, die auf eine kompliziertere Art vererbt werden, und zu berücksichtigen, dass es mehr als eine soziale Schicht gibt. Formeln dieser Art können selbstverständlich auch für Berechnungen über die Wirkung der Selektion angewandt werden, die eine Rolle für die Beschaffenheit der selektiven Isolate in der untersten Schicht einer Gesellschaft spielen, wobei man erwarten kann, dass die Selektion auf Eigenschaften gerichtet ist, die aus sozialen Gesichtspunkten als ungünstig anzusehen sind und sich auf mässige Intelligenz, kriminelle Tendenzen, mangelhafte Selbstbeherrschung und ähnliches beziehen.

In zweiter Linie wurde mit diesen Berechnungen nur beabsichtigt zu zeigen, dass uns faktisch Unterlagen für eine rationelle Besprechung der Probleme fehlen. In der ständigen Diskussion über die soziale Organisation und bei der Aufstellung der Utopien, die eine grössere oder kleinere Rolle für das politische Handeln spielen, üben Vorstellungen über erblich bedingte Unterschiede zwischen den Gesellschaftsklassen einen bedeutenden Einfluss aus. Für diese Besprechung muss es ein Vorteil sein, dass man sich klar macht, dass Anhaltspunkte für bestimmte Behauptungen fehlen und dass es deswegen von Bedeutung ist, die Diskussion auf andere Ausgangspunkte als Annahmen über das Vorhandensein oder Fehlen von ausgeprägten erblichen Unterschieden zwischen den verschiedenen Gesellschaftsklassen aufbaut. Es kann für die Diskussion auch einen Vorteil darstellen, eine Vorstellung darüber zu erhalten, welche Bedeutung eine rationelle Selektion innerhalb einer Gesellschaft spielen

könnte, die radikal auf eine andere Weise organisiert wäre, als die, in der wir leben.

Schliesslich kann es von Bedeutung sein, Berechnungen vorzunehmen, bei denen man von der Annahme ausgeht, dass man eine ideale Rekrutierung der Oberklasse unter dem Gesichtspunkt der Vererbung erreicht hat. Wir nehmen an, dass man eine individuelle Prüfung vornimmt, ehe die Individuen die Ausbildung erhalten, die immer mehr notwendig ist, um in die leitende Schicht einer Gesellschaft aufzusteigen. Wir nehmen ferner an, dass die Tests, die ausgeführt werden, soweit zuverlässig sind, dass keine Personen, welchen die Eigenschaft fehlt, die man zu erhalten wünscht, in die Oberklasse aufsteigen. Schliesslich nehmen wir an, dass die Grösse der Oberklasse dem vorhandenen Bedarf an einer leitenden Schicht angepasst wird und dass deswegen eine grössere oder kleinere Gruppe dieser speziellen Eigenschaftsträger dem Oberklassenisolat angehören wird. Dies bedeutet, dass in den Formeln 87, 88 und 89  $k_2 = 0$ . Unter solchen Umständen sieht die Formel für die Zusammensetzung der Unterklasse folgendermassen aus:

$$r_{a(n+1)}^2 = k_1 r_n^2 \quad (92)$$

Die Formel für die Oberklasse nimmt folgende Form an:

$$r_{b(n+1)}^2 = \frac{(r - k_1 r_n^2)^2}{1 - k_1 r_n^2} \quad (93)$$

TABELLE 30.

Beschaffenheit der Totalpopulation, wenn diese in eine Oberklasse und eine Unterklasse eingeteilt ist und wenn die Eigenschaftsträger in verschiedener Frequenz und in verschiedenem Umfang der Oberklasse zugeführt werden.

Vgl. Formel 94.

Genera- tion	$r^2 = 0.000001$		$r^2 = 0.0001$		$r^2 = 0.01$	
	$k = 0.25$	$k = 0.50$	$k = 0.25$	$k = 0.50$	$k = 0.25$	$k = 0.50$
0	0.000001000	0.000001000	0.0001000	0.0001000	0.01000	0.01000
1	0.000001250	0.000001499	0.0001245	0.0001490	0.01203	0.01407
2	0.000001312	0.000001748	0.0001305	0.0001730	0.01244	0.01574
3	0.000001327	0.000001872	0.0001320	0.0001848	0.01250	0.01642
4	0.000001331	0.000001934	0.0001323	0.0001907	0.01254	0.01671
5	0.000001332	0.000001965	0.0001324	0.0001936	0.01255	0.01682
6		0.000001981		0.0001951		0.01687
7		0.000001989		0.0001956		0.01689
8		0.000001993		0.0001959		0.01690
9		0.000001995		0.0001960		

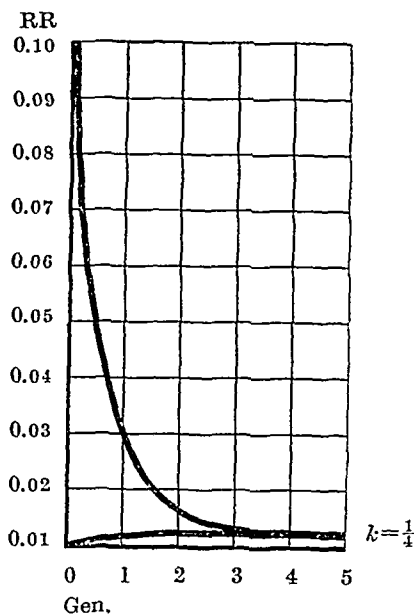


Abb. 31. Frequenz von Eigenschaftsträgern in einer Population, wenn eine monohybride rezessive Eigenschaft die Frequenz 1 % hat und  $\frac{1}{4}$  der Eigenschaftsträger der Oberklasse zugeführt wird. Die Berechnungen sind ausgeführt ausgehend von Panmixie und auch unter der Annahme, dass in der Population auf Grund von totaler Gattenwahl keine Heterozygoten existieren.

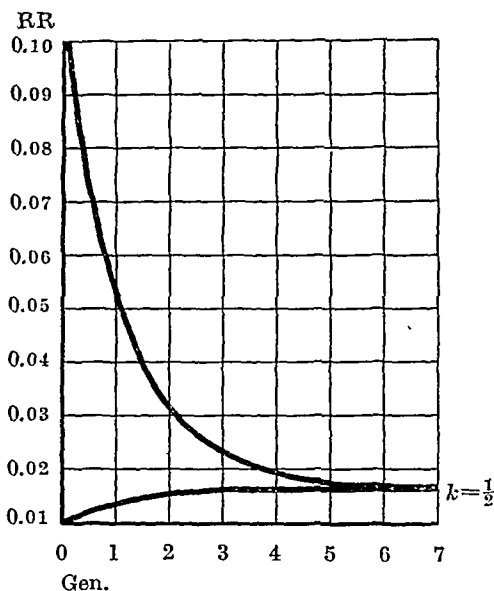


Abb. 32. Frequenz von Eigenschaftsträgern in einer Population, wenn eine monohybride rezessive Eigenschaft die Frequenz 1 % hat und  $\frac{1}{2}$  der Eigenschaftsträger der Oberklasse zugeführt wird. Die Berechnungen sind ausgeführt ausgehend von Panmixie und auch unter der Annahme, dass in der Population auf Grund von totaler Gattenwahl keine Heterozygoten existieren.

Für die ganze Bevölkerung bekommen wir folgende Rekursionsformel:

$$r_{n+1}^2 = k_1 r_n^2 + \frac{(r - k_1 r_n^2)^2}{1 - k_1 r_n^2} \quad (94)$$

Um den Grenzwert zu erreichen, kann man so zu Wege gehen wie früher bei partieller Gattenwahl, d. h. man geht bei den Berechnungen teils von Panmixie, teils von einer Bevölkerung aus, in der totale Gattenwahl stattgefunden hat, so dass die Heterozygoten verschwunden sind und sich nur die beiden Homozygotenarten vorfinden. Wir finden dann, dass falls die Auswahl eine sehr seltene monohybrid rezessive Eigenschaft betrifft, die z. B. die Frequenz 0.01 % hat, und falls  $\frac{1}{4}$  der Eigenschaftsträger dem Oberklassen-



isolat angehören wird, die Frequenz der genannten Eigenschaft in der Bevölkerung etwas zunehmen und nach 3—4 Generationen eine Gleichgewichtslage bei 0.013 % erreichen wird. Bei einer etwas häufiger vorkommenden rezessiven Eigenschaft, die die Frequenz 1 % hat, erreicht man, wenn die Hälfte der Eigenschaftsträger dem Oberklassenisolat angehören, nach 7—8 Generationen eine Gleichgewichtslage. Man erhält eine Zunahme der Eigenschaftsträgerfrequenz in der Bevölkerung von 1 % auf 1.7 %. (Vgl. Tabelle 30 und Abb. 31 und 32.) Die Berechnungen haben im Augenblick geringes praktisches Interesse, man kann aber die Möglichkeit nicht von der Hand weisen, dass sie in der Zukunft eine Bedeutung bekommen können.

## Zusammenfassende Übersicht.

Man kann behaupten, dass die medizinische Erbllichkeitsforschung zwei Ziele besitzt. Das eine ist, *theoretisch* zu ermitteln versuchen, welche Rolle Vererbung und Milieu spielen, und zu verstehen versuchen, was psychisch und physisch unsere Eigenschaften bedingt. Für die Erbllichkeitsforschung existieren nur Vererbung und Milieu. Etwas Drittes gibt es nicht. Mit anderen Worten, es wird mehr oder minder bewusst ein absoluter Determinismus vorausgesetzt. Das Ziel ist weder grösser noch geringer als das Problem des Lebens insofern zu lösen zu versuchen, als ob wir nach Erlangung erschöpfender Kenntnisse über die Erbfaktoren und Milieuverhältnisse, die ein Individuum bedingen, dieses derart »verstehen« würden, dass wir in der Lage wären, es in seinen ursächlichen Zusammenhang einzureihen.

Das zweite Ziel ist *praktischer* Art. Es gilt zu erforschen, was Vererbung und Milieu für einzelne Individuen und Populationen bedeuten, und vor allem müssen Voraussagen über die Beschaffenheit der Individuen, die geboren werden, gemacht werden. Es handelt sich dabei darum zu untersuchen, unter welchen Umständen die Erbmasse eines Volkes konstant ist, und wie sie unter der Einwirkung von speziellen Faktoren sich in der einen oder der anderen Richtung erwartungsgemäss verändert. Handelt es sich um einzelne Individuen, so liegen dieselben Aufgaben vor, nämlich Angaben zu verschaffen versuchen, welche eine Voraussage über die Beschaffenheit des Nachkommens erlauben, welcher aus einer Verbindung zwischen einzelnen Personen (über die man so vollständige Angaben als nur möglich besitzt) hervorgeht. Selbstverständlich besitzt es von beiden Gesichtspunkten aus an und für sich ein grösseres Interesse zu erforschen, welche Rolle Vererbungsmomente für gewöhnlichere Eigenschaften als für seltenere spielen. Indessen muss auch berücksichtigt werden, dass seltene Eigenschaften vom sozialen Gesichtspunkt aus als besonders bedeutungsvoll erscheinen können,

und zwar auf Grund ihres Charakters. Für die Gesellschaft bedeutet ein Individuum mit einer Erbbeschaffenheit, welche eine extrem niedrige oder extrem hohe Effektivität bedingt, mehr als ein Individuum mit sog. normalen Eigenschaften. Deswegen sind Probleme über Anlagen, welche Defekte bzw. exzeptionelle Begabung bedingen, von besonderem Interesse.

Will man Individuen bewerten, so muss man selbstverständlich auch zwischen individuellen und sozialen Gesichtspunkten unterscheiden. Von dem eigenen Gesichtspunkt des Individuums aus betrachtet (sowie von dem von Personen, die ihm nahestehen) besitzt sein Leben insoweit einen unbegrenzten Wert, als es unersetzlich ist, was ja nicht hindert, dass einzelne Individuen andere Dinge höher bewerten können als das Leben. Gewisse Zielsetzungen und Vorstellungen darüber, was gerecht oder gut ist, können ja dazu führen, dass Individuen in gewissen Situationen willig sind, ihr Leben zu opfern. Hierbei liegt ja eine grosse Variation vor, und zwar nicht nur von Individuum zu Individuum, sondern auch zwischen verschiedenen Populationen und vor allem zwischen verschiedenen Zeitperioden.

Will man Individuen vom sozialen Gesichtspunkt aus bewerten, so ist es am nächsten liegend, eine ökonomische Betrachtungsweise anzuwenden. Ein Individuum besitzt dann keinen Wert, wenn die Kosten, welche seine Erziehung und sein Dasein bedingen, dem ökonomischen Beitrag, welchen seine Arbeitsleistungen während des Lebens ausmachen, exakt entsprechen. Das Individuum hat einen negativen Wert, wenn die genannten Unkosten das, was es leisten kann, überschreiten, und einen positiven Wert in dem entgegengesetzten Fall. Man hat den Versuch gemacht, von diesen Ausgangspunkten aus ökonomische Berechnungen auszuführen, und diese besitzen unzweifelhaft ein gewisses Interesse. Offensichtlich besitzen die zahlenmässigen Werte ein grösseres Interesse, falls es sich um Individuen mit Defekten handelt, die einen negativen Wert besitzen, und zwar auch dann, wenn in zivilisierten Ländern nur vereinzelte Personen geneigt sein dürften, hierbei konsequent einen rein ökonomischen Gesichtspunkt anzulegen und andere Bewertungen als bedeutungslos anzusehen. Handelt es sich um Personen mit einer eher durchschnittlichen Beschaffenheit, so ist die Berechnung u. a. mit der Schwäche behaftet, dass man auf Grund der variierenden Arbeitslosigkeit gegenwärtig nicht entscheiden kann, zu einer wie um-

fangreichen Arbeitsleistung eine Person, die willig ist zu arbeiten, während ihres Lebens in der Tat Möglichkeit erhält. Handelt es sich um exzeptionell begabte Individuen, so ist das Problem schliesslich noch schwerer zu lösen, da man keine Anhaltspunkte besitzt, um den Wert von deren Leistungen vom ökonomischen Gesichtspunkt aus zu bewerten. Dasselbe gilt selbstverständlich auch bezüglich Personen mit einem durchschnittlicheren Intelligenzniveau, die in einem Land zufallsweise eine leitende Stellung erlangt haben.

Es ist deshalb offensichtlich, dass Begabungen einen sehr unterschiedlichen Typus aufweisen und sehr unterschiedlich bewertet werden können. Einige sind z. B. der Ansicht, dass die künstlerische Begabung am wichtigsten ist, andere schätzen das logische Denken und Kombinationsvermögen und wieder andere nehmen an, dass ein starker Wille, kombiniert mit einer Rücksichtslosigkeit von höherem oder geringerem Grad, wertvoller ist als alles andere.

Innerhalb der menschlichen Erbllichkeitsforschung wurde allmählich eine grosse Anzahl von Detailuntersuchungen ausgeführt. Nach der Wiederentdeckung der Gesetze von MENDEL um die Jahrhundertwende herum hat man sich darauf konzentriert zu beweisen, dass diese Gesetze auch für den Menschen Gültigkeit besitzen. Es ist am leichtesten, dies bezüglich seltener Krankheiten, welche bis zu einem gewissen Grade den Charakter von Kuriositäten besitzen, zu zeigen. Deswegen hat man sich vor allem für solche Eigenschaften interessiert. Es war ferner selbstverständlich, dass man versucht hat, die experimentelle Vererbungsforschung zu kopieren. Die Untersuchungen erhielten deshalb oft den Charakter einer Erforschung von Familien, in welchen mehrere Fälle mit einer seltenen Eigenschaft vorgekommen sind. Das Resultat wurde in Form von Stammbäumen aufgezeichnet. Ausgehend von den Vorvätern wurde festgestellt, dass unter den Nachkommen in einer oder mehreren Generationen Eigenschaften in der Weise aufgetreten sind, wie man es nach dem Gesetz von MENDEL erwarten kann. Nebenbei sei bemerkt, dass die Methode, die man angewandt hat, fehlerhaft war. Man ist nicht, wie innerhalb der experimentellen Vererbungsforschung, von einer bestimmten Kreuzung ausgegangen und untersuchte, welche Beschaffenheit die Nachkommenschaft erhält. Man hat anstatt dessen die Ausgangspersonen ausgewählt, da man die Beschaffenheit der Nachkommenschaft kannte. Die Antwort auf die Frage, die man gestellt hat, war mit anderen Worten bis zu einem gewissen

Grad gegeben, und die Analogie mit den experimentellen Untersuchungen war tatsächlich nur eine scheinbare. Es ist deshalb nicht immer leicht, das Material, welches eingesammelt wurde, zu bewerten. Es ist schwer und mitunter unmöglich zu entscheiden, welche Rolle das Auswahlmoment gespielt hat.

Der Umstand, dass man beim Menschen keine Kreuzungsversuche ausführen kann, sondern sich damit begnügen muss, die Kreuzungen zu analysieren, die in menschlichen Populationen vorkommen, bedingt es, dass wir in der menschlichen Erbllichkeitsforschung andere Methoden anwenden müssen als in der experimentellen. Die speziellen Verhältnisse beim Menschen bringen es ferner mit sich, dass andere Fragestellungen interessieren. Es ist erwünscht, dass man bei der Planierung von Detailuntersuchungen die Probleme und Fragestellungen bis zu einem gewissen Grade berücksichtigt, die in der vorliegenden Arbeit angedeutet wurden. Es ist mit anderen Worten ein wünschenswertes Ziel, dass die menschliche Erbllichkeitsforschung nicht allzu sklavisch denselben Linien folgen soll wie die experimentelle Erbllichkeitsforschung, wenn es auch notwendig ist und immer notwendig sein wird, dass zwischen den beiden Forschungsrichtungen ein bestimmter Kontakt aufrecht erhalten wird.

Es ist nun möglich, die Probleme theoretisch und mathematisch herauszuarbeiten, welche vom Standpunkt der Populationen aus innerhalb der menschlichen Erbllichkeitsforschung dem Interesse am nächsten liegen. Es zeigt sich dabei, dass gegenwärtig empirische Anhaltspunkte für die Lösung einer ganzen Reihe sehr bedeutungsvoller Probleme fehlen. Es fehlen uns Angaben über das Vorkommen von Verwandtschaftsehen und Gattenwahl. Wir wissen sehr wenig über Isolatgrenzen und Umstellungen innerhalb von Populationen u. s. w. Es dürfte aber nicht auf unüberwindliche Schwierigkeiten stossen, in diesen Punkten unser Wissen zu komplettieren, nachdem man zur Klarheit darüber gelangt ist, dass solche Angaben eine wesentliche Bedeutung haben. Auch wenn die mathematische Analyse von Populationen vom Gesichtspunkt der Vererbung aus noch in vielen Punkten mangelhaft ist und auch wenn die empirischen Unterlagen für eine solche Analyse äusserst spärlich sind, kann es doch motiviert sein, bereits in diesem frühen Stadium zu versuchen, eine Übersicht über die Ergebnisse und Problemstellungen zu geben, die sich bisher ergeben haben, und sie ausgehend von gewissen empirischen Ausgangspunkten in Kürze zu besprechen.

Der Ausgangspunkt für unsere Analyse ist gewesen, dass eine Person vom Standpunkt der Vererbung aus sechs verschiedene Möglichkeiten hat. Sie kann sich *erstens* zufallsbedingt verheiraten (und fortpflanzen). Wird auf diese Weise die Ehe geschlossen, so sagt man, dass eine Panmixie vorliegt. Die Person kann *zweitens* es sein lassen, sich zu verheiraten, oder sie pflanzt sich in geringerem Ausmasse als der Durchschnitt fort. Handelt eine Gruppe von Eigenschaftsträgern auf diese Weise, so liegt eine Selektion vor. *Drittens* kann ein Eigenschaftsträger eine Verwandtschaftsbeziehung eingehen. Kommen Verwandtschaftsbeziehungen in grösserem Ausmasse vor, als sie zufallsbedingt zu erwarten sind, so spricht man davon, dass eine Inzucht vorliegt. *Viertens* kann eine Person eine grössere Neigung dazu haben, sich mit Eigenschaftsträgern einer bestimmten Art zu verheiraten als mit anderen. Die wichtigste Möglichkeit hierbei ist, dass Eigenschaftsträger einer bestimmten Art eine grössere Neigung haben sich mit einander zu verheiraten als es der Zufall erfordert. Dies wird „assortative mating“ oder Gattenwahl genannt. Eine Person, die sich verheiratet, hat *fünftens* keine Möglichkeit sich über die Grenzen einer ganzen Population hinaus zu verheiraten. Ihre Bewegungsfreiheit wird durch bestimmte geographische und soziale Verhältnisse begrenzt. Sie verheiratet sich innerhalb einer bestimmten Region und einer bestimmten Klasse, und es besteht eine geringere Aussicht für sie sich ausserhalb der Gesellschaftsklasse und der Grenzen der Region zu verheiraten. Dies bedeutet, dass in einer Population Isolatgrenzen vorhanden sind. *Sechstens* hat man mit der Möglichkeit zu rechnen, dass eine Person, die sich verheiratet, Nachkommen erhält, die auf Grund einer Mutation von dem abweichen, was man zu erwarten hat. Diese verschiedenen Möglichkeiten schliessen selbstverständlich einander nicht aus. Wählt eine Person eine andere zur Ehe, kann sie also zufallsbedingt wählen, überhaupt nicht wählen, eine verwandte Person wählen, innerhalb eines bestimmten Isolates wählen, einen Eigenschaftsträger einer bestimmten Art wählen, und schliesslich kann sich in die Wahl eine Mutation einmischen. Geschehnisse dieser letzteren Art können es bedingen, dass Abweichungen von dem entstehen, was man bei Panmixie zu erwarten hat, d. h. bedingen, dass kommende Generationen eine von früheren abweichende Beschaffenheit annehmen. Liegt hingegen eine Panmixie vor, so ist die Erbbeschaffenheit der Bevölkerung von Generation zu Generation konstant.

Es ist in erster Linie von Interesse festzustellen, dass durch Selektion und Mutation die Erbmasse eine Veränderung durchmachen kann, während hingegen Verwandtschaftsehe, Isolatwirkung und Gattenwahl die Erbmasse nicht verändern, sondern nur bedingen, dass Gene in grösserem oder geringerem Ausmasse als bei Panmixie zu erwarten ist, zu Homozygotie zusammengeführt werden. Diese letzteren Prozesse bewirken keine Veränderung des Gengehaltes der Population und wenn eine Panmixie eintritt, nachdem solche Prozesse eine Zeit lang stattgefunden haben, so erhält man nach einer kürzeren oder längeren Zeit erneut die Zusammensetzung der Population, die man vor Beginn der Prozesse hatte. Wenn auch theoretisch gesehen die Veränderungen, die Verwandtschaftsehe, Gattenwahl und die Isolatwirkung bedingen, reversibel sind, so bedeutet dies indessen nicht, dass es in menschlichen Populationen immer möglich ist die Wirkung solcher Prozesse aufzuheben und auf einen früheren Zustand zurückzugehen.

*Selektion* und *Mutationen* ändern also nicht nur die Frequenz der Homozygoten und Heterozygoten in einer Population. Diese Prozesse beeinflussen auch den Gengehalt der Population, so dass, falls man sich vorstellt, dass die Prozesse nach einer Zeit aufgehoben werden und eine Panmixie wieder eintritt, man eine andere Zusammensetzung der Population erhalten würde, als man sie im Ausgangs- augenblick hatte. Es fehlen uns exakte Angaben darüber, in welcher Frequenz Mutationen vorkommen, es besteht aber am ehesten Anlass anzunehmen, dass diese sehr selten sind, was bedeutet, dass die Veränderungen, die Mutationen möglicherweise bedingen können, sehr langsam vorsichgehen und kaum feststellbar eine Population im Laufe weniger Generationen beeinflussen. Mutationen besitzen deswegen für die menschliche Erbllichkeitsforschung weniger Interesse. Anders dagegen, wenn man das Problem auf sehr lange Sicht betrachtet, z. B. wenn man sich mit dem Evolutionsproblem auf dem Gebiete der Zoologie und Botanik beschäftigt.

Eine totale Selektion rottet augenblicklich monohybride, dominante Eigenschaftsträger aus. Bei partieller Selektion ist die Geschwindigkeit, mit welcher der Prozess verläuft, von der Stärke der Selektion abhängig.

Dasselbe gilt für konditionelle Eigenschaften, wenn die Anlage dominant ist und eine totale Selektion vorliegt. Bei einem solchen Erbgang pflegt man von einer Dominanz mit einer bestimmten Ma-

nifestationswahrscheinlichkeit zu sprechen. Mancherorts wird der Ausdruck Penetranz hierfür angewandt. Diese Bezeichnungen werden aber mitunter nur angewandt, um auszudrücken, dass auf Grund unbekannter Faktoren eine bestimmte Anlagekombination nur bei einem Teil der Fälle eine bestimmte Eigenschaft hervorruft. Die Ursache können Milieufaktoren, aber auch bestimmte Erbanlagen sein, die nicht immer vorkommen, sondern nur mit einer bestimmten Frequenz. Auf Grund dieser Unklarheit kann Anlass bestehen diese Ausdrücke zu vermeiden. Sie geben keine klare Vorstellung darüber, was die verschiedenen Autoren mit ihnen bezeichnen und geben in vielen Fällen einen falschen Eindruck, nämlich dass man weiss, worüber man spricht, wenn in Wirklichkeit nur Anlass besteht hervorzuheben, dass bestimmte Behauptungen nicht aufgestellt werden können. — Das hauptsächliche Interesse richtet sich auf monohybride, rezessive Eigenschaften oder auf solche, die auf eine kompliziertere Art vererbt werden, da ja schwere Defekte, die sich frühzeitig geltend machen, nicht dominant vererbt werden können. Die Selektion hat ihre grösste Bedeutung für rezessive Eigenschaften, wenn sie häufig vorkommende Eigenschaften betrifft. Sie kann dann, auch wenn sie schwach ist, in kurzer Zeit wesentliche Verschiebungen bewirken. In dem Masse wie Selektionsprozesse in früheren Generationen vorgekommen sind, ist es wahrscheinlich, dass die Selektion ihre hauptsächliche Wirkung ausgeübt hat und dass sie nicht länger wesentlichere Verschiebungen verursacht. Hingegen ist es selbstverständlich von Interesse zu ermitteln zu versuchen, ob Selektionsprozesse späteren Datums möglicherweise beginnen, sich in menschlichen Populationen geltend zu machen.

Eine Selektion, die während einer grossen Anzahl von Generationen gewirkt hat, muss bewirkt haben, dass die Eigenschaften, die sie betroffen hat, auf eine sehr niedrige Frequenz herabgepresst wurden. Theoretisch gesehen hat man dann zu erwarten, dass die Frequenz der Eigenschaft sich der Gleichgewichtslage nähert, in der den Eigenschaftsträgern, welche durch die Selektion ausgerottet werden, neu hinzugekommene Mutationen entsprechen. Diese Grenze nennen wir kleinste Heterozygotengrenze, um zu betonen, dass in der Gleichgewichtslage eine verhältnismässig hohe Frequenz der Heterozygoten vorhanden ist, die zufällig zusammentreffen und Anlass zu vereinzelter Eigenschaftsträgern werden. Diese stammen praktisch genommen immer von normalen Eltern ab, die Heterozy-



goten sind. Diese Situation muss im Hinblick auf die schweren vererbaren Defekte vorliegen, die sich bei der Geburt oder vor dem geschlechtsreifen Alter geltend machen. Es ist mit anderen Worten nicht möglich, durch besondere Massnahmen, wie z. B. Sterilisierung, die Frequenz von Eigenschaften wesentlich herabzusetzen, die eine niedrige Fruchtbarkeit bedingen. Ein Beispiel für eine Eigenschaft, die sich ungefähr in der Gleichgewichtslage befindet, da die Eigenschaftsträger äussert selten sind, wo aber eine verhältnismässig grosse Anzahl von Heterozygoten für die Eigenschaft in der Population vorhanden ist, stellt die juvenile amaurotische Idiotie dar. In Schweden werden jährlich 4—5 solche Eigenschaftsträger geboren, die regelmässig gegen das geschlechtsreife Alter hin sterben und sich niemals fortpflanzen — eine effektivere Selektion als Sterilisierung — und in Schweden sind etwa 50 derartige Eigenschaftsträger vorhanden (SJÖGREN 1931).

Es soll im übrigen daran erinnert werden, dass eine Selektion, die rezessive Eigenschaften betrifft, auch wenn sie absolut ist, doch nur asymptotisch die Frequenz der Eigenschaft 0 näher bringt, was bedeutet, dass die Eigenschaft in endlicher Zeit nicht vollständig ausgerottet werden kann. Um dies zu illustrieren, soll daran erinnert werden, dass bei monohybrider Rezessivität jedem Eigenschaftsträger, der daran verhindert wird, sich fortzupflanzen, zwei Heterozygoten entsprechen, die, falls die Anlage sehr selten ist, den beiden heterozygoten Eltern entsprechen. Dies bedeutet, dass falls die Bevölkerung pro Generation verdoppelt wird, die Anzahl der Heterozygoten in der Bevölkerung (nicht aber deren Frequenz) unverändert bleibt.

Handelt es sich um schwere erbliche Defekte, muss daran erinnert werden, dass seit sehr fernen Zeiten solche Eigenschaftsträger kaum die Möglichkeit gehabt haben, sich fortzupflanzen. Starben die Eltern, so waren sie auf eine praktisch genommen nicht vorhandene Wohltätigkeit angewiesen. Soziale Gesichtspunkte und ein humanes Handeln sind in der menschlichen Geschichte erst sehr spät aufgetreten, und auch die jetzt lebenden Generationen sind nur in sehr geringem Ausmasse von derartigen Gesichtspunkten nennenswert beeinflusst. Die Hilfe, die auch in den zivilisiertesten Ländern defekten Individuen geleistet wird, besitzt keine solche Grössenordnung, dass sie es diesen Individuen ermöglicht, sich frei fortzupflanzen. Früher lebten sie am Rande der Not, und ihre Fortpflanzung war

sicher sehr gering. Ausgehend von diesen Gesichtspunkten kann man erwarten, dass die Frequenz an erblichen Defekten in den verschiedenen Ländern keine grossen Unterschiede aufweisen wird, und dass man auch in Bevölkerungen, die auf einem sehr primitiven Niveau leben, finden wird, dass die Defekte eine Frequenz von ungefähr derselben Grössenordnung aufweisen. Eventuelle Verschiedenheiten in der Frequenz müssen ein Ausdruck für die verschiedene Mutationsfrequenz sein. Leider fehlen uns zuverlässige Angaben, die eingehendere Vergleiche ermöglichen.

Wenn die Selektion ohne grössere Bedeutung für seltene, erbliche Defekte ist, muss sie auch ohne nennenswerte Bedeutung sein, wenn sie seltene vorteilhafte Eigenschaften, wie hohe Begabung, betrifft. Man kann möglicherweise behaupten, dass die katholische Kirche unter vielen Generationen an einer Selektion mitgewirkt hat, die gegen hohe Begabung gerichtet war. In älteren Zeiten hatten begabte Personen, die nicht der Oberklasse angehörten, praktisch genommen nur eine Möglichkeit, intellektuelle Arbeit zu erhalten, nämlich die, Priester zu werden. Hierdurch wurde die Begabung Gegenstand einer Selektion, da es auf Grund des Zölibates für Priester nicht erlaubt war, Kinder zu haben. Trotzdem liegt auf jeden Fall kein deutlicher Unterschied zwischen der Frequenz an hoher Begabung in den katholischen Ländern im Vergleich zu anderen vor. Wenn in einem Land die Herrschenden während einer kürzeren oder längeren Zeit die selbstständig Denkenden, die einen integrierenden Bestandteil an exzeptioneller Begabung darstellen, nicht tolerieren, und deswegen versuchen solche Individuen auszurotten, so kann es ein Trost sein zu wissen, dass dies freilich das kulturelle Niveau des Landes gewiss beeinflussen, aber nicht bewirken kann, dass die Eigenschaft verschwindet oder ihre Frequenz überhaupt nennenswert verändert.

Wenn auf Grund von Verwandtschaftsruhe, Gattenwahl oder Isolawirkungen die Homozygotie in einer Bevölkerung verändert wird, kann die Gleichgewichtslage, die zwischen den Mutationen und der Selektion vorliegt, aufgehoben werden. Durch Isolataufhebung kann z. B. die Homozygotie vermindert werden. Dies bewirkt, dass die kleinste Heterozygotenfrequenz verschoben wird. Es liegt dann nicht länger ein Gleichgewicht zwischen den Mutationen und der Selektion vor. Es zeigt sich, dass auch eine mässige Verschiebung der Gleichgewichtslage es bedingt, dass es eine unendlich lange Zeit dauert, ehe die Gleichgewichtslage erneut erreicht wird, und man

kann berechnen, dass es etwa hundert Generationen dauert, bevor ein kleinerer Unterschied zur Hälfte ausgeglichen wird. Dasselbe gilt, wenn durch eine verminderte Anzahl von Verwandtschaftsehen die Gleichgewichtslage verändert wird. Letale und subletale Gene sowie Gene, die schwere angeborene Defekte und ähnliches bedingen, werden in einer solchen Situation eine grössere Verbreitung erhalten, und falls z. B. Verwandtschaftsehen nach mehreren tausend Jahren erneut eingeführt werden, so würde dies eine erhöhte Frequenz solcher Eigenschaften bewirken, insofern sie rezessiv sind oder auf eine kompliziertere Art vererbt werden. Man könnte sich vorstellen, dass dies eine Gefahr darstellt. Man könnte geltend machen, dass Prozesse, die auf diese Weise die Gleichgewichtslage zwischen Selektion und Mutationen stören, unvorteilhaft sind. An und für sich sind aber selbstverständlich Anlagen gleichgültig, so lange sie latent sind, und deswegen bedeuten auf jeden Fall auf kürzere Sicht Prozesse, die eine erhöhte Heterozygotie bedingen, einen Vorteil. In welchem Ausmasse man der Ansicht sein soll, dass der augenblickliche Gewinn durch eine sehr fragliche Ungelegenheit in der Zukunft aufgehoben werden wird, ist letzten Endes eine subjektive Frage.

Nehmen wir an, dass man durch eine verminderte Heterozygotie die Möglichkeit erhalten könnte, dass während einiger Generationen eine effektive Selektion zustande kommen könnte und dass später automatisch eine erhöhte Heterozygotie mit einer konstant niedrigeren Frequenz erblich bedingter Defekte auftreten würde, als es der Frequenz in der Gleichgewichtslage entspricht, die früher zwischen Selektion und Mutationen vorhanden war. Die Antwort auf die Frage, inwieweit man diesen Weg beschreiten soll, ist natürlich davon abhängig, in welchem Ausmasse man der Ansicht ist, dass sich die jetzt lebenden Generationen für kommende opfern sollen. Die Persönlichkeiten, die sich für Vererbungshygiene interessieren, geben nicht selten der Ansicht Ausdruck, dass man alles für kommende Geschlechter opfern muss, während man im allgemeinen sich im sozialen Handeln im grossen und ganzen in der Art des Handelns von Gesichtspunkten, die sich auf sehr kurze Sicht beziehen, und von den Interessen der jetzt Lebenden bestimmen lässt. Es besteht selbstverständlich keine Möglichkeit, objektiv die Interessen der jetzt Lebenden gegenüber denen kommender Generationen abzuwägen. Würde man davon ausgehen, dass den Interessen der ein-

zelnen Personen dasselbe Gewicht zuzumessen ist, und zwar unabhängig von dem Zeitpunkt, in welchem sie leben, müssten die jetzt Lebenden jederlei Last tragen, wenn sie hierdurch einen unbedeutenden Vorteil für kommende Generationen gewinnen könnten. Setzt man voraus, dass die Menschheit während einer beträchtlichen Zeit weiter leben wird, muss ja die Summe des Gewinnes für die unzähligen kommenden Generationen beliebig grosse Ungelegenheiten einer oder einiger früherer Generationen aufwiegen. Stellt man sich hingegen vor, dass eine Population kleiner werden wird und verschwindet, muss man billigerweise, wenn alle Individuen als gleichwertig angesehen werden, in bestimmten Fällen die Lasten auf die kleineren, zukünftigen Generationen schieben. In der Wirklichkeit ist es selbstverständlich eine Geschmacksache, wie viel jetzt lebende Generationen für kommende opfern sollen. Man kann in diesem Punkt keine objektive Beurteilung motivieren. Im allgemeinen dürfte die gewöhnliche Einstellung die sein, dass je weiter man die Schwierigkeiten von sich schieben kann, es desto besser ist, und dies nicht am wenigsten aus dem Gesichtspunkt, dass man hoffen darf Möglichkeiten zu finden, den Schwierigkeiten zu entgehen, die wir jetzt noch nicht einmal ahnen können.

Im Hinblick auf die Frequenz, die eine Eigenschaft auf Grund einer bestimmten Tendenz zu Mutationen erreichen kann, muss daran erinnert werden, dass eine Anlage, die durch Mutation entstanden ist, nicht selten zufallsbedingt verschwindet. Die Wahrscheinlichkeit hierfür ist u. a. von der Familiengrösse abhängig. Entsteht eine Anlage und ist bei einer Person in heterozygoter Form vorhanden, so ist natürlich die Gefahr, dass die Anlage in der nächsten Generation nicht repräsentiert werden wird, in einer Bevölkerung grösser, in der die Fruchtbarkeit klein ist, als in einer, in der sie gross ist. In Wirklichkeit hat man nicht nur damit zu rechnen, dass Mutationen in grossem Ausmasse zufallsbedingt verschwinden, sondern auch damit, dass sie zufallsbedingt an Zahl zunehmen. Es trifft selbstverständlich leichter in kleinen Isolaten ein, dass eine Anlage eine höhere Frequenz erreicht als in grossen Isolaten. Sind die Isolate klein, so hat man damit zu rechnen, dass bestimmte Anlagen in einer Reihe von Isolaten vollständig fehlen und in anderen Isolaten zufallsbedingt eine grössere oder kleinere Frequenz haben.

Ebenso wie man mancherorts die Neigung gehabt hat, die Bedeutung der Selektion zu überwerten (vor allem in Form der Sterili-

sierung), war man auch geneigt, *Verwandtschaftsehen* eine allzu grosse Wirkung zuzuschreiben. Man muss sich indessen daran erinnern, dass streng genommen alle Ehen Verwandtschaftsehen sind, insoweit man eine einheitliche Abstammung für das menschliche Geschlecht annimmt (oder eine Abstammung von einer kleinen Gruppe von Individuen). Dies ergibt sich u. a. daraus, dass wenn man die Anzahl der Vorfäter für eine Person zeitlich nach rückwärts berechnet und annimmt, dass diese nicht mit einander verwandt waren, so erhält man sehr schnell astronomische Zahlen, die weit die Anzahl der Individuen überschreiten, die auf der Erde gelebt haben. Man kann deswegen eine bestimmte Bevölkerung mit einer bestimmten Frequenz an Verwandtschaftsehen nicht mit einer theoretisch angenommenen Bevölkerung vergleichen, in welcher keinerlei Verwandtschaftsehen vorkommen. Es ist angebracht, zum Ausgangspunkt für einen Vergleich eine Bevölkerung mit Panmixie anzunehmen, in welcher Verwandtschaftsehen zufallsbedingt vorkommen. Ist die Bevölkerung gross, so wird die Frequenz näherer Verwandtschaftsehen praktisch genommen null sein.

Dass man Verwandtschaftsehen überwertet, dürfte teilweise mit dem Umstand zusammenhängen, dass man innerhalb der experimentellen Erbliehkeitsforschung durch Generation nach Generation wiederholte Geschwisterkreuzungen eine sehr kräftige Wirkung erhalten hat. Die Heterozygoten verschwinden praktisch genommen nach einer verhältnismässig geringen Anzahl von Generationen.

Beim Menschen ist die Vetternehe die stärkste Verwandtschaftsehe, mit der man in der Praxis zu rechnen hat. Diese hat nur eine geringe Wirkung auf eine gewöhnliche Eigenschaft. Ist eine Eigenschaft selten, so besitzt sie eine nicht unwesentliche Bedeutung, die Eigenschaft spielt aber dann eine kleine Rolle in der Bevölkerung. Kommt eine rezessive Eigenschaft bei 25 % der Bevölkerung bei Panmixie vor und würden plötzlich nur zwischen Vettern und Basen Ehen geschlossen, so würde in der nächsten Generation die Frequenz der Eigenschaft auf 26.56 % erhöht werden. Ist die Eigenschaft hingegen sehr selten, wenn die Anlage z. B. ein einziges Mal durch Mutation entstanden ist, so können Eigenschaftsträger nur durch Verwandtschaftsehen entstehen, und Vetternehen müssen dann eine sehr wichtige Rolle spielen. Sind Vetternehen sehr häufig und die Anlage sehr selten, dürfte man durch ein Verbot von Vetternehen die Frequenz der Eigenschaft wesentlich herabsetzen können, insofern es sich um

eine rezessive Eigenschaft oder eine Eigenschaft handelt, die auf eine kompliziertere Art vererbt wird. (Bei Dominanz bewirken Verwandtschaftsehen, dass die Frequenz der Eigenschaft unbedeutend abnimmt.) Nachdem die Population in Isolate aufgeteilt ist, kann man aber nicht sowohl eine hohe Frequenz an Verwandtschaftsehen als auch seltene Anlagen erhalten. In einem Isolat müssen mindestens zwei Heterozygoten vorhanden sein, damit ein Eigenschaftsträger entstehen kann. Ist das Isolat gross, kann die Anlage eine niedrige Frequenz haben, aber dann sind kaum Vetternehen häufig vorkommend. Ist das Isolat klein, ist die Frequenz an Vetternen höher, die Anlage kann aber dann nicht extrem selten sein. Diese Verhältnisse bringen die Wirkung der Vetternehen ins Gleichgewicht, so dass man in der Praxis maximal damit zu rechnen hat, dass 15—30 % der Eltern der Eigenschaftsträger Vetter und Base sind. Dies stimmt mit den Ergebnissen überein, die man bei der Untersuchung seltener Eigenschaften erhalten hat. Bei juveniler amaurotischer Idiotie sind 15 % der Eltern Vetter und Base. Bei Retinitis pigmentosa ist der entsprechende Wert 17 %, bei Friedreichs Ataxie 9 %, bei Albinismus 17 % u. s. w. Durch ein Verbot von Vetternehen würde man mit anderen Worten eine grössere Wirkung im Hinblick auf seltene erbliche Defekte erhalten als möglicherweise durch Sterilisierungsmassnahmen. Alle selteneren rezessiven oder auf komplizierte Weisen erblichen Eigenschaften würden gleichzeitig beeinflusst werden, und der gesamte Effekt würde sicherlich nicht unbedeutend sein. Es würde sich aber um einen einmaligen Effekt handeln. Man würde in folgenden Generationen nichts gewinnen.

Man würde nun befürchten können, dass man durch ein Verbot der Vetternehen auch eine Herabsetzung seltener vorteilhafter Eigenschaften, wie extreme Begabung einer bestimmten Art u. s. w., erhalten würde. Eigenschaften dieser Art dürften indessen nicht wie monohybride Eigenschaften vererbt werden, sondern einen verwickelteren Erbgang aufweisen. Die Wirkung auf diese Eigenschaften wird deshalb auf jeden Fall weniger stark. Dass man in einer Reihe von Ländern Vetternehen verboten hat, dürfte indessen kaum mit vererbungshygienischen Gesichtspunkten etwas zu tun haben. Die Illusionsbildung, die in gewissem Masse die Voraussetzung für die Ehe ist, stellt sich nicht so leicht Personen gegenüber ein, die nahe Verwandte sind und die man gut kennt. Auf Grund dessen sind Ehen zwischen Vettern und Basen vielleicht als unnatürlich betrach-

tet worden. Erst viel später hat man versucht, die Handlungsweise zu rationalisieren. Will man aus vererbungshygienischen Gesichtspunkten ein Verbot der Vetternehen diskutieren, so muss man selbstverständlich berücksichtigen, dass eine solche Massnahme einen Eingriff in die Handlungsfreiheit des Einzelnen bedeutet, der an und für sich nicht gleichgültig ist. Geht man davon aus, dass der Staat den Mitgliedern der Gesellschaft eine so grosse Freiheit als möglich geben und nur dann eingreifen soll, wenn zwingende Gründe vorliegen, so ist natürlich die Massnahme auf jeden Fall diskutabel. Mancherorts hat man einen grossen Eifer gezeigt, vererbungsbiologische Ergebnisse praktisch anzuwenden, von denen sich später gezeigt hat, dass sie schlecht begründet waren. Dies mahnt zur Vorsicht.

Der Umstand, dass Verwandtschaftsehen für die Frequenz aller seltenen Eigenschaften, die rezessiv oder auf eine kompliziertere Art vererbt werden, Bedeutung haben, bedingt es, dass Verwandtschaftsehen eine Korrelation zwischen seltenen Eigenschaften verursachen. Geht man von Trägern seltener Eigenschaften aus, wählt man in besonders grossem Ausmasse Nachkommen aus Vetternehen. Man hat deswegen zu erwarten, dass bei diesen und unter deren Geschwistern eine erhöhte Frequenz anderer seltener Eigenschaften vorhanden ist. Dies dürfte zum Teil die Erklärung dafür sein, dass Missbildungen verschiedener Art verhältnismässig oft im Kreise von Geschwistern zusammentreffen und dass man auch bei anderen seltenen Eigenschaften ein auffallendes Zusammentreffen seltener Defekte gefunden hat (z. B. Taubstummheit und Retinitis pigmentosa). Eine Korrelation zwischen Eigenschaften kann ja auf einer Koppelung beruhen, kann aber auch durch Verwandtschaftsehen bedingt sein, was früher nicht beachtet wurde. Man hat schliesslich mit der Möglichkeit zu rechnen, dass seltene Eigenschaften in kleinen Isolaten eine höhere Frequenz haben. Dies bedingt eine Korrelation zwischen Eigenschaften ähnlicher Art wie die, welche die Verwandtschaftsehe verursacht.

In Wirklichkeit liegt, wie bereits weiter oben hervorgehoben, zwischen Isolat und Verwandtschaftsehe ein Zusammenhang vor. Eine Population ist in Teilpopulationen aufgeteilt, wobei man annehmen kann, dass innerhalb derselben Panmixie vorliegt. Diese *Isolate* können geographisch begrenzt oder sozial abgegrenzt sein. In bestimmten Fällen handelt es sich aber um scharfe Isolatgrenzen, in

anderen Fällen sind die Grenzen unscharf; selbstverständlich kommt es auch vor, dass keine eigentlichen Grenzen vorhanden sind. Abgesehen davon hat eine bestimmte Person eine grössere Aussicht, sich mit Personen zu verheiraten, die in einem geringeren oder mässigen Abstand von ihr leben, und praktisch genommen keine Aussicht, sich mit Personen zu verheiraten, die weit entfernt von ihr leben. Es liegt eine Isolierung vor, aber keine scharfen Isolatgrenzen.

Man kann nun Vetternehen dazu verwenden, um eine Vorstellung über die Isolatgrösse zu erhalten. Sind die Isolate klein, so haben Vetternehen eine hohe Frequenz. Sind die Isolate gross, ist die Frequenz an Vetternehen klein. Mit Hilfe der Vetternehen kann man ein schematisiertes Isolat berechnen, das die Anzahl von Individuen umfasst, die bei einer bestimmten Familiengrösse einer gegebenen Frequenz an Vetternehen entsprechen würde. Man ersetzt dann eine variierende Wahrscheinlichkeit für die Ehe mit einer durchschnittlichen. Die Frequenz an Vetternehen hat in zahlreichen Ländern gegen die jetzige Zeit wesentlich abgenommen, was mit der Industrialisierung, der Flucht in die Städte und den besseren Kommunikationen zusammenhängen dürfte. Man kann berechnen, dass dies einer Zunahme der durchschnittlichen Isolatgrösse von 200 Personen bis zu 800 in einer Reihe von Ländern Westeuropas entspricht.

Man kann indessen nicht davon ausgehen, dass Vetternehen unter allen Umständen dem entsprechen, was man bei Panmixie zu erwarten hat. In Ländern, in welchen die Eheschliessungen durch die Eltern bestimmt werden, muss die Frequenz an Vetternehen höher sein, als man sie auf Grund der Isolatgrösse erwarten würde. Sie muss ferner in geringerem Ausmasse durch Isolataufhebung beeinflusst werden, als es in anderen Bevölkerungen der Fall ist; sie muss aber nicht völlig unempfindlich sein, da die Isolataufhebung doch eine herabgesetzte Möglichkeit für die Mitglieder eines Geschlechtes für die Aufrechterhaltung des gegenseitigen Kontaktes bedeuten muss. Es ist möglich, dass die verhältnismässig hohe Frequenz an Vetternehen in Frankreich mit sozialen Momenten dieser Art zusammenhängt. Unter solchen Umständen ist es von Interesse, zuverlässigere und differenziertere Angaben über Vetternehen in verschiedenen Populationen zu erhalten.

Schliesslich soll hervorgehoben werden, dass eine Zunahme der Frequenz an Vetternehen unter den Eltern von Eigenschaftsträgern einen wichtigen Leitfaden für die Beurteilung der Bedeutung



der Vererbungsmomente darstellt und dass man bisher in allzu geringem Ausmasse diese Möglichkeit benutzt hat. Tritt z. B. eine Krankheit verhältnismässig oft bei den Eltern und Geschwistern kranker Personen auf, so kann dies auf Milieumomenten beruhen (Ansteckung). Eine Erhöhung der Frequenz an Vetternehen kann kaum mit etwas anderem als Vererbungsmomenten zusammenhängen. Beim Vergleich muss man natürlich den Wert für Vetternehen anwenden, der die Isolate charakterisiert, aus welchen man die Eigenschaftsträger genommen hat.

Es ist gewissermassen eine Geschmacksache, ob man, wenn man die Isolataufhebung diskutiert, diese als das Wesentliche ansehen will oder das Hauptgewicht auf die verminderte Frequenz an Verwandtschaftsehen legen will. Wenn man auch mit Hilfe der Frequenz an Vetternehen Anhaltspunkte für eine Beurteilung der Isolatgrösse und der Isolataufhebung erhalten kann, so fehlen uns doch gegenwärtig direkte Angaben anderer Art für die Beurteilung des Grades der Isolierung. Es ist aber möglich, dass man Angaben erhalten könnte, die hierbei einen Leitfaden geben könnten. Eine Voraussetzung ist selbstverständlich, dass man in der menschlichen Erblichkeitsforschung die hier angedeuteten Gesichtspunkte, die bisher kaum bekannt waren, anlegt.

Um über das Verhältnis zwischen Isolat und Verwandtschaftsehe zu orientieren, stellen wir uns ein geschlossenes Isolat, z. B. eine Insel vor, auf welcher die Population schnell an Grösse zunimmt. Dies bedeutet, dass falls eine Panmixie vorliegt, die Frequenz an Vetternehen abnimmt. Ungeachtet dessen, wird die Frequenz an Eigenschaftsträgern nicht verändert werden. Dies beruht darauf, dass entferntere Grade von Verwandtschaftsehen an Frequenz zunehmen, und dies gleicht die verminderte Frequenz an Vetternehen aus. Könnte man Verwandtschaftsehen entfernteren Grades registrieren, würde es möglich sein, den Prozess nur vom Standpunkt der Verwandtschaftsehe aus zu betrachten. Da dies indessen nicht möglich ist, ist es geeigneter, den Prozess im Hinblick auf das Isolat zu analysieren. Dieser Gesichtspunkt gilt für eine Reihe von Problemen, denen eine sehr wesentliche Bedeutung zukommt.

Die Isolatgrenzen haben nun für die Heterozygotenfrequenz keine Bedeutung, wenn die Populationen in den verschiedenen Isolaten dieselbe Beschaffenheit haben. Haben hingegen bestimmte Eigenschaften in einer Reihe von Isolaten eine höhere Frequenz, eine nied-

rigere in anderen, so wird man, wenn man von den Isolatgrenzen absieht, ausgehend von den Eigenschaftsträgern, einen höheren Gehalt an Heterozygoten annehmen, als er faktisch vorliegt. In dem extremen Falle, dass in zwei Isolaten die eine Anlage bei allen Personen des einen Isolates vorhanden ist und in dem anderen vollständig fehlt, in dem also keine Heterozygoten vorkommen, würde man dann radikal falsche Schlüsse ziehen, falls man bei dem Versuch, sich eine Vorstellung über den Anlagegehalt der Population zu bilden, von den Isolatgrenzen absehen würde.

Handelt es sich um seltene Eigenschaften, so hat man in Wirklichkeit wesentliche Unterschiede zwischen den Isolaten zu erwarten. Diese besitzen natürlich eine sehr variierende Grösse in einer Population. Das eine Extrem stellen die grossen Städte dar, das andere kleine Dörfer in Gebieten mit Einödecharakter. Entstehen in einer Population Mutationen, so können diese zufallsbedingt deswegen verschwinden, weil sich die betreffende Person nicht fortpflanzt u. s. w. oder zufallsbedingt eine mässige Anzahl von Heterozygoten in späteren Generationen bewirken. Ist die Anlage rezessiv, kann sie sich nicht geltend machen und wird frühestens in der vierten Generation Gegenstand einer Selektion, da Verwandtschaftsehen näheren Grades sehr selten sind und Geschwisterehen nicht vorkommen. Die Wahrscheinlichkeit dafür, dass eine Mutation zufallsbedingt eine mässige Frequenz erreicht, ist selbstverständlich in einem kleinen Isolat weit grösser als in einem grossen. Deswegen findet man seltene Defekte vor allem in kleinen Isolaten, in abgelegenen Dörfern, in welchen in der Regel verhältnismässig primitive Verhältnisse herrschen und in welchen Vetternehen und andere Verwandtschaftsehen eine hohe Frequenz haben. Man pflegt nicht selten geltend zu machen, dass die defekten Individuen in Isolaten und die primitiven kulturellen Verhältnisse eine durch Verwandtschaftsehen verursachte Degeneration darstellen. Dies ist selbstverständlich falsch. An und für sich besteht kein Anlass zu der Annahme, dass die Erbmasse der Bevölkerung in kleinen Isolaten weder besser noch schlechter als in grösseren Isolaten ist, abgesehen von der besonders hohen Frequenz des mutierten Genes. Das Wesentliche des Prozesses stellt nicht die Verwandtschaftsehe dar; eine grössere Population mit demselben Gengehalt, aber weniger Vetternehen, würde dieselbe Frequenz an Eigenschaftsträgern aufweisen. Das Wesentliche stellen die engen Isolatgrenzen dar, die

durch den Mangel an Kommunikationen u. s. w. bedingt werden. Auf Grund der verbesserten Kommunikationen, der Flucht in die Städte u. s. w. hat, wie oben erwähnt, die Frequenz an Vetternehen abgenommen, was einen Ausdruck dafür darstellt, dass die Isolatgrenzen beseitigt oder erweitert wurden. Man kann die Sache auch so ausdrücken, dass auf Grund dieser Prozesse die Isolate grösser geworden sind. Handelt es sich um seltene Eigenschaften, kann man davon ausgehen, dass falls diese in einem Isolat vorhanden sind, es kaum wahrscheinlich ist, dass dieselbe Anlage in angrenzenden Isolaten zu finden sein wird. Durch Kreuzungen über die Isolatgrenzen hinaus, wird die Frequenz der Eigenschaftsträger vermindert. Anlagen werden in heterozygoter Form verbreitet. Dieser Prozess geht im extremen Fall mit der Vergrösserung des Isolates proportional. An Hand der Frequenz der Vetternehen kann, wie eben erwähnt, berechnet werden, dass die Isolate in einer Reihe westeuropäischer Länder während einiger Dezennien an Grösse verdoppelt und möglicherweise verdreifacht worden sind, was dazu geführt haben muss, dass die Frequenz an seltenen Defekten wesentlich abgenommen hat, ohne dass deswegen die Frequenz der Gene, die den Defekten entsprechen, vermindert wurde. Dies muss einen Vorteil bedeuten, da der Prozess irreversibel ist. Legt man das Hauptgewicht in diesem Prozess auf die verminderte Anzahl von Verwandtschaftsehen, so beurteilt man die Situation falsch, insoweit man nur stärkere Grade von Verwandtschaftsehen berücksichtigt. Die Frequenz an Eigenschaftsträgern hat wahrscheinlich weit mehr abgenommen, als es der Abnahme der Vetternehen entspricht. Wenn aus irgend einem Anlass die Frequenz an Vetternehen in der Zukunft zunehmen würde, muss dies nicht bedingen, dass die Frequenz der Eigenschaftsträger zunimmt und das ältere Niveau erreicht.

In der offiziellen Statistik findet man aber nicht die Abnahme an defekten Personen, die gemäss dieser Überlegungen zu erwarten wäre. Es muss aber daran erinnert werden, dass die Registrierung derselben immer vollständiger geworden ist. Abgesehen davon ist diese Registrierung noch weit davon entfernt, zufriedenstellend zu sein. Schweden wird als Land mit einer relativ zuverlässigen Bevölkerungsstatistik angesehen. Unabhängig davon betrug z. B. die Epilepsiefrequenz in Schweden bei der letzten Volkszählung 1910 nur 4349. Man ist in der Regel nicht gern bereit, den Behörden Angaben über Defekte zu machen. Hierbei ist aber eine Ausnahme vorhanden,

nämlich die militärische Musterung. An Hand der Angaben von dieser kann berechnet werden, dass die Anzahl der Epileptiker in Schweden ungefähr 12 000 betragen muss, was bedeutet, dass ein grosser Spielraum für eine erhöhte Frequenz durch eine verbesserte Registrierung vorhanden ist, wenn auch die wirkliche Frequenz erblicher Epilepsie wesentlich zurückgehen würde.

Auf Grund dessen, dass die Isolate auf dem Lande kleiner sind als in den Städten, muss man eine höhere Frequenz erblicher Defekte unter der Landbevölkerung, verglichen mit der Stadtbevölkerung, erwarten. Der Unterschied muss indessen zum Teil darauf beruhen, dass bei der Einwanderung in die Städte eine Selektion stattfindet. Die Defekten bleiben in verhältnismässig grossem Ausmasse auf dem Lande zurück. Da aber Personen mit rezessiven Defekten so gut wie ausschliesslich von Eltern abstammen, die Heterozygoten sind, muss sich dieser Umstand nur in der letzten eingewanderten Generation geltend machen. Die Heterozygoten in früheren Generationen müssen ebenso zahlreich sein wie in der ursprünglichen Bevölkerung.

Im Hinblick hierauf kann es von Interesse sein, die zahlenmässigen Angaben vom Lande und aus Städten, sowie aus einigen verschiedenen Gebieten Schwedens miteinander zu vergleichen. Die Werte stammen von der Volkszählung 1930 her. Vgl. Tabelle 31.

TABELLE 31.

Frequenz defekter Individuen in ‰ in verschiedenen Verwaltungsgebieten Schwedens laut der Volkszählung 1930.

Verwaltungsgebiet	Blinde	Taub- stumme	Geistes- schwache	Geistes- gestörte	Epilep- tiker	Krüppel
Stockholm .....	0.86	0.49	1.23	3.93	0.37	8.57
Stockholm län.....	0.67	0.74	2.69	3.66	0.65	10.99
Uppsala län.....	0.98	0.99	3.17	4.00	0.82	11.81
Södermanland län .....	0.86	0.73	2.91	4.32	0.83	11.41
Malmöhus län .....	0.49	0.69	2.28	4.44	0.71	9.20
Västerbotten län.....	1.52	1.45	3.62	4.47	0.77	11.71
Norrbotten län .....	1.32	1.15	3.26	4.53	0.99	11.36
Schwedens Städte .....	0.73	0.74	1.62	4.51	0.50	9.51
• Landgemeinden	1.10	0.93	3.46	4.54	0.81	11.68
Ganz Schweden	0.98	0.87	2.86	4.53	0.71	10.97

Bei der Beurteilung dieser Werte muss man sich daran erinnern, dass nur ein bestimmter Teil dieser Defekte erblich bedingt ist. Gegenwärtig fehlt uns die Möglichkeit, im Einzelfalle immer bestimmt zu entscheiden, ob die Defekte milieubedingt sind oder nicht. Ferner soll hervorgehoben werden, dass die Rubriken, die angewandt werden, eine Anzahl verschiedener erblich bedingter Defekte umfassen, wie Blindheit, Idiotie, Verkrüppelung u. s. w. Sie sind also teils aus einer Anzahl durch verschiedene Gene bedingter Defekte und teils aus milieubedingten Defekten zusammengesetzt. Die Registrierung ist schliesslich, wie oben hervorgehoben, mangelhaft. Unter solchen Umständen muss man sich damit begnügen festzustellen, dass man Frequenzunterschiede findet, die im grossen und ganzen dem entsprechen, was man unter Berücksichtigung der Isolatgrösse zu erwarten hat. Die Frequenz an defekten Personen ist in den Städten niedriger als auf dem Lande und extrem niedrig in Stockholm sowie extrem hoch in den am weitesten nördlich gelegenen Teilen des Landes (Västerbotten, Norrbotten), wo die Isolate verhältnismässig klein sind. (Dass im Hinblick auf die Geistesgestörten keine nennenswerten Unterschiede vorliegen, dürfte damit zusammenhängen, dass diese in weit grösserem Ausmasse als auf dem Lande in den Städten gepflegt und registriert werden.) Es muss auf jeden Fall von Interesse sein zu versuchen, die Werte für die Frequenz der Defekte in den verschiedenen Gebieten zu analysieren, und zwar unter Anwendung der hier angegebenen Gesichtspunkte.

Zusammenfassend soll im Hinblick auf die seltenen Defekte hervorgehoben werden, dass man durch Sterilisierung kaum erwarten kann, ihre Frequenz in nennenswertem Ausmasse zu beeinflussen. Eine grössere Wirkung würde man durch ein Verbot der Vetternehen erhalten. Der Prozess, der die grösste Bedeutung besitzt, ist die Isolataufhebung. Mancherorts hat man auf dem Gebiete der Vererbungshygiene hervorheben wollen, dass die Landbevölkerung der Kern des Volkes ist, und man hat u. a. mit dieser Motivierung versucht, Massnahmen zu ergreifen, um den Zuzug zu den Städten zu verhindern und die Bevölkerung auf dem Lande zurückzuhalten. Denkt man hierbei an die Frequenz defekter Personen, so ist diese Ansicht falsch. Defekte haben die grösste Frequenz in den verhältnismässig kleinen Isolaten auf dem Lande und will man ihre Frequenz vermindern, muss man einen Zuzug in die Städte befürworten.

Nun ist aber die Frequenz an defekten Personen keineswegs von entscheidender Bedeutung für eine Population. Auch wenn man ihnen eine menschenwürdige Existenz bieten würde, würden die Kosten unbedeutend sein, nicht zum wenigsten verglichen mit den unerhörten, luxusbetonten Ausgaben, welche der organisierte Massensmord auf Grund des Krieges bedeutet. Von weit grösserer Bedeutung sind selbstverständlich die Leiden, welche die Defekte für die Eigenschaftsträger und ihre Eltern bedingen.

Auf dem Gebiete der Vererbungshygiene spricht man vielerorts von der Frequenz der Defekte, als ob diese eine unerhört grosse Bedeutung für die allgemeine Effektivität besitzen würde. In Wirklichkeit bedeuten selbstverständlich das allgemeine intellektuelle Durchschnittsniveau und die Frequenz exzeptionell Begabter viel mehr für eine Gesellschaft.

Im Hinblick auf die Frequenz der besonders Begabten ist, wie bereits oben erwähnt, die Selektion ohne nennenswerte Bedeutung, insoweit man diese so abgrenzt, dass sie eine seltene Eigenschaft ist. Was man auch unter besonderer Begabung versteht, so dürfte man ferner davon ausgehen können, dass eine grössere oder kleinere Frequenz von Verwandtschaftsehen keine nennenswerte Rolle für die Frequenz der Eigenschaft spielt, insofern man an Vetternehen und entsprechende nähere Grade von Verwandtschaftsehen denkt. Dies deswegen, weil die Eigenschaft in dem Grade, in welchem sie erblich bedingt ist, sicher durch mehrere Gene erzeugt wird, die zusammentreffen müssen. Für solche Eigenschaften besitzt eine grössere oder geringere Frequenz von Vetternehen eine geringe Bedeutung. Hingegen ist es möglich, dass die Isolatgrenzen für solche Eigenschaften eine wesentliche Bedeutung besitzen. Um dies zu veranschaulichen, soll in Kürze die Bedeutung der Isolataufhebung für die Körperlänge besprochen werden. Zwischen Begabung und Körperlänge liegen vom Standpunkt der Vererbung aus wesentliche Ähnlichkeiten vor. Die Körperlänge in einer Population weist eine ziemlich normale Verteilung mit einer niedrigen Frequenz an extrem kleinen und grossen Personen sowie einer hohen Frequenz an Personen mittlerer Grösse auf. Eine ähnliche Verteilung hat man bei der Ausführung sog. Intelligenzteste erhalten. Unterhalb der normalen Verteilung der Variationsbreite für die Körperlänge findet man eine kleinere Anzahl extrem kleiner Personen. Die Ursache hierfür ist bei einem Teil der Fälle eine Milieuschädigung, bei anderen Fällen eine besondere

Erbanlage (Zwerge, Kyphose auf Grund von Tuberkulose u. s. w.). Analog hierzu findet man unterhalb der Dummheit, die nach unten die normale Begabung abgrenzt, eine kleinere Anzahl von Individuen, die entweder auf Grund von Milieuschädigungen (wie z. B. Gehirnblutungen bei der Geburt) oder auf Grund besonderer Erbanlagen ausgeprägt defekt sind.

In zahlreichen Ländern konnte man eine Zunahme der Körperlänge bis in die jetzige Zeit feststellen. In Schweden beträgt die Zunahme für 100 Jahre 9 cm. Es liegt am nächsten anzunehmen, dass diese durch einen erhöhten Lebensstandard bedingt wird. In Wirklichkeit verläuft aber die Zunahme der Körperlänge nicht mit der Erhöhung des Lebensstandards parallel. Es besteht Anlass anzunehmen, dass eine bessere Ernährung die Geschwindigkeit beeinflusst, mit welcher jüngere Personen ungefähr bis zur Pubertät wachsen. Hernach geht das Wachstum bei guter Ernährung langsamer vor sich, so dass im erwachsenen Alter wahrscheinlich kein nennenswerter Unterschied vorliegt. Man hat zeigen können, dass von 1883—1938/39 der Unterschied in der Körperlänge für Personen im Alter von 15 Jahren in Schweden ungefähr 15 cm, für Erwachsene aber nicht ganz 5 cm ist. Wird die Körperlänge durch mehrere Gene bestimmt, die vorwiegend dominant sind, ist es möglich, dass die Isolataufhebung, die in Schweden im Gange ist und die eine erhöhte Heterozygotie bedingt hat, die wesentliche Ursache dafür darstellt, dass die Körperlänge bei Erwachsenen zugenommen hat. Verf. hat dieses Problem in mehreren Arbeiten besprochen (vgl. DAHLBERG 1931 und 1938, BROMAN, DAHLBERG und LICHTENSTEIN 1942).

Die Isolataufhebung würde also im Hinblick auf die Körperlänge zu einer Verminderung der Anzahl extrem kleiner Personen, einer Zunahme der Durchschnittsgrösse und einer Zunahme der Anzahl extrem grosser Personen führen. Läge für intellektuelle Begabung ein ähnlicher Prozess vor, dürfte man erwarten, dass die Isolataufhebung eine Abnahme der Frequenz extrem Dummer, eine höhere Durchschnittsintelligenz und eine erhöhte Frequenz extrem begabter Personen bewirkt hat.

Um die Bedeutung der Isolataufhebung im Hinblick auf die normale Intelligenz näher zu untersuchen, sind Untersuchungen darüber erforderlich, wie sich Minderbegabung vererbt. Findet man, dass sich Minderbegabung als eine komplizierte rezessive Eigenschaft vererbt, so besteht Anlass zu der Annahme, dass die Isolataufhebung

ein vom Standpunkt der Gesellschaft aus vorteilhafter Prozess ist. Im entgegengesetzten Fall stellt sie eine Gefahr dar. Diese Seite der Sache ist möglicherweise wichtiger als die Wirkung, welche die Isolataufhebung auf die Frequenz erblich defekter Personen gehabt hat und noch hat.

Theoretisch gesehen besteht selbstverständlich die Möglichkeit, dass der Prozess der Isolataufhebung gleichgültig ist und dass rezessive und dominante Anlagen sich auf eine solche Art das Gleichgewicht halten, dass bei Isolataufhebung keine Verschiebungen vorkommen.

In dem Masse wie die Isolataufhebung die erblichen Eigenschaften in einer Population beeinflusst, hat man Unterschiede zwischen der Stadt- und der Landbevölkerung zu erwarten. Die Heterozygotie ist in der Stadtbevölkerung grösser. Prozesse dieser Art können ferner Unterschiede zwischen verschiedenen Ländern bewirken. Die Heterozygotie ist selbstverständlich in einem Land, das seit alter Zeit bewohnt ist, geringer als in einem Land, in welches eine Einwanderung stattgefunden hat und eine Besiedlung vom Kolonisationstypus vorliegt. Es muss z. B. in diesem Punkt ein Unterschied nicht nur zwischen Europa und Amerika, sondern auch zwischen Amerika und England vorliegen.

Auf dem Gebiete der Vererbungsforschung müssen mit anderen Worten Fragen, die sich auf die Isolataufhebung beziehen, einen wichtigen Platz einnehmen, und es ist in erster Linie wichtig, Primärangaben über den Umfang des Prozesses in verschiedenen Ländern zu erhalten.

Handelt es sich um *Gattenwahl*, so fehlen uns ebenfalls Primärangaben, die einer Analyse über die Bedeutung solcher Prozesse zugrunde gelegt werden können. Mathematisch konnte gezeigt werden, dass die Gattenwahl sehr schnell bis zu einer Gleichgewichtslage führt. Es ist deswegen vor allem von Interesse zu untersuchen, in welchem Ausmasse Prozesse dieser Art in der letzten Zeit vor sich gegangen sind. Die verbesserten Kommunikationen haben solche Prozesse in grösserem Ausmasse ermöglicht. Handelt es sich um seltene Eigenschaften, so kann Gattenwahl kaum irgend eine grössere Rolle spielen, da ja die seltenen Eigenschaftsträger gerade auf Grund ihrer Seltenheit keine grossen Möglichkeiten haben zusammenzutreffen. Die Isolataufhebung hat aber die Möglichkeit für einen solchen Prozess erhöht. In welchem Ausmasse Gattenwahl für gewöhn-



liche Eigenschaften vorkommt, ist bisher kaum untersucht worden. Man weiss aber, dass im Hinblick auf die Körperlänge eine solche Tendenz vorliegt. Grosse bzw. kleine Personen verheiraten sich etwas häufiger miteinander als es zufallsbedingt zu erwarten ist.

Theoretisch gesehen ist es möglich, dass für bestimmte Eigenschaften eine negative Gattenwahl vorkommt. Es ist vor allem wahrscheinlich, dass bestimmte Gebrechen, die Hilflosigkeit bedingen, auf Ehen zwischen Personen, welche dieselbe Eigenschaft haben, hemmend wirken. Prozesse dieser Art müssen eine schwache Zunahme der Heterozygotie bewirken, es ist aber unwahrscheinlich, dass solche Prozesse in menschlichen Populationen eine feststellbare Wirkung haben.

In der Wirklichkeit dürfte die Gattenwahl vor allem in Zusammenhang mit Isolatbildung vorkommen. Defekte Personen, z. B. Taubstumme, bilden Isolate, die mehr oder minder scharf abgegrenzt sind. Man hat z. B. zu erwarten, dass Taubstumme in der Mehrzahl der Ehen beide den Defekt aufweisen, was im Hinblick auf die niedrige Frequenz der Taubstummen in der Population eine sehr starke Gattenwahl bedeutet. Das Isolat der Taubstummen setzt sich nun aus erblich Taubstummen und Personen zusammen, die an milieubedingter Taubstummheit leiden.

Bei etwa der Hälfte der Taubstummen ist das Leiden erblich bedingt. Einige Taubstumme verheiraten sich ausserhalb der Isolatgrenze und gehören dem speziellen Defektisolat, welches durch Taubstumvereine oder ähnliche Organisationen gebildet wird, überhaupt nicht an. Die Lage ist in verschiedenen Populationen verschieden. Augenblicklich ist es nicht möglich, mit den hier angegebenen Formeln Berechnungen auszuführen. Durch empirische Untersuchungen könnte man möglicherweise Konstanten erhalten, welche solche Berechnungen erlauben könnten.

Man kann vermuten, dass ähnliche Prozesse im Hinblick auf erbliche Anlagen, die spezielle Interessen bedingen, vorliegen. Z. B. musikalische Personen treffen sich in einem bestimmten Ausmass in besonderen Vereinen und Bekanntenkreisen, so dass man mit einer gewissen Berechtigung von selektiven Isolaten sprechen könnte. Gleichzeitig muss man auch damit rechnen, dass das Musikinteresse auch in Gesellschaften, in welchen musikalische Isolate kaum vorhanden sind, eine Gattenwahl bedingt. Untersuchungen, die von den hier angegebenen Gesichtspunkten ausgehen, wurden nicht ausgeführt.

In dem Masse, in welchem eine Tendenz zur Gattenwahl im Hinblick auf seltene Eigenschaften, z. B. erblich bedingte Defekte oder ausserordentliche Begabung vorliegt, hat man Anlass zu vermuten, dass die Isolataufhebung bedingt, dass diese jetzt eine etwas grössere Rolle spielt als es früher der Fall war. Dadurch dass die Isolate grösser geworden sind, haben die Träger seltener Eigenschaften grössere Möglichkeiten bekommen, zusammenzutreffen. Der Prozess bedingt eine Zunahme der Homozygotie und eine Abnahme der Heterozygotie und wirkt also der Isolataufhebung und der Abnahme an Verwandtschaftsgrad entgegen. Es ist nicht möglich, die Wirkung dieser Prozesse exakt abzuwägen, man dürfte aber am ehesten Anlass haben zu vermuten, dass die eventuelle Zunahme der Gattenwahl keine wesentlichere Rolle spielen wird. Es ist aber erwünscht, dass empirische Untersuchungen über dieses Problem ausgeführt werden. Die Verhältnisse sind natürlich für verschiedene Eigenschaften unterschiedlich. Handelt es sich um Taubstumme, hat man z. B. Anlass anzunehmen, dass die Isolataufhebung eine erhöhte Möglichkeit für diese bedingt, zusammenzutreffen. Dadurch, dass solche Personen gleichzeitig eine verbesserte Ausbildung erhalten haben, welche ihre Möglichkeiten für einen Kontakt mit normalen Personen erhöht haben, ist es aber möglich, dass die Tendenz zu einer Gattenwahl gleichzeitig abnimmt. Das Ergebnis dieser Prozesse kann nur durch empirische Untersuchungen festgestellt werden.

Die Form der Gattenwahl, die durch besonders eingestellte Interessen bedingt wird, führt zur Frage nach der Bedeutung der sozialen Klassengrenzen vom Standpunkt der Vererbung aus. Personen, die in der einen oder anderen Gesellschaftsklasse geboren wurden und durchschnittliche Eigenschaften haben, verbleiben in der Mehrzahl der Fälle innerhalb ihrer Gesellschaftsklasse. Es findet aber eine gewisse Zirkulation zwischen den Ständen statt, was teils eine Versetzung von Personen mit durchschnittlicherem Charakter von einem Stand in einen anderen, teils auch eine selektive Umstellung bedeutet. Personen mit Eigenschaften, die eine besondere soziale Effektivität bewirken, haben eine gewisse Aussicht, in eine höhere Gesellschaftsklasse zu gelangen. Besonders minderbegabte Personen sinken in niedrigere Gesellschaftsklassen herunter. Man kann selbstverständlich auch hier von Isolat mit einer selektiven Überführung von Personen sprechen, man kann aber auch das Gewicht auf spezielle, eventuell erblich bedingte Interesseneinstellungen richten und die

Ansicht haben, dass eine Gattenwahl über die Isolatgrenzen hinaus stattfindet. Man hat vielleicht eher Anlass, den letztgenannten Standpunkt zu betonen, wenn es sich um Frauen handelt, und bezüglich der Männer das Selektionsmoment hervorzuheben. In dem Ausmasse, in welchem sich Frauen direkt in die Oberklasse hinein verheiraten, ist es möglich, dass es sich um eine positive Gattenwahl im Hinblick auf die Intelligenz, aber vielleicht vor allem um eine negative Gattenwahl im Hinblick auf das Aussehen handelt. Arbeitet man sich in die Oberklasse herauf, haben die Männer ja oft eine Ehe geschlossen, ehe sie die Isolatgrenzen überwinden konnten. Die Frauen, die auf diese Weise den Oberklassenisolaten zugeführt werden, haben die für die Unterklasse durchschnittliche Beschaffenheit, insoweit für die Gruppe von Männern, die sich heraufarbeitet, keine Gattenwahl vorliegt.

Im Hinblick auf die Männer, die in die Oberklasse gelangen, ist selbstverständlich eine besondere Einstellung der Interessen konstitutiv. Ohne einen auf dieses Ziel eingestellten Willen wird die Grenze kaum überschritten. Es erhebt sich nun die Frage, in welchem Ausmasse besondere erbliche Eigenschaften von Bedeutung sind. Zufallsmomente spielen selbstverständlich eine nicht unwesentliche Rolle. Unter im übrigen gleichen Umständen muss aber besondere Begabung ein Vorteil sein, ebenso aber auch ein harter Wille, eine bestimmte Egozentrität und Rücksichtslosigkeit. Es ist möglich, dass die Personen, die sich heraufarbeiten, in gewissem Ausmasse eine Auswahl von Personen darstellen, die vorteilhafte intellektuelle und unvorteilhafte moralische Eigenschaften haben.

Mathematisch ist es möglich die Wirkung der selektiven oder assortativen Isolatbildung zu berechnen, welche die sozialen Klassengrenzen bedeuten, wenn man den Charakter und die Grösse der Umstellung kennt, die stattfindet. Man kann zeigen, dass eine Auswahl im Hinblick auf besondere erbliche Eigenschaften eine fortschreitende Verschiebung der Beschaffenheit des Oberklassenisolates bewirken muss. Ob die Wirkung ausreichend gross ist um eine Rolle zu spielen, kann aber auf Grund unseres bisherigen Wissens nicht entschieden werden. Da die Oberklasse verhältnismässig klein ist und auch die Standes-zirkulation gering ist, kann man aber behaupten, dass durch eine eventuelle Auswahl die breiten Schichten ihren Charakter nicht verändern. Für die niedrigeren Gesellschaftsschichten kann man die Ansicht haben, dass es sich um eine Selektion handelt, die



klasse auszeichnen. Man sieht es deswegen als für die Gesellschaft von Interesse an, dass die Begabten für eine unbestimmte Zeit in der Unterklasse zurückgehalten werden. (Man gibt keinen Zeitpunkt an, in welchem die Gesellschaft ihre Begabung anwenden kann.) Gleichzeitig macht man geltend, dass die niedrige Fruchtbarkeit in der Oberklasse einen Ausdruck für eine moralische Minderwertigkeit, für einen sozialen Egoismus, darstellt. Man wünscht eine höhere Fruchtbarkeit unter diesen Asozialen.

Wir haben hier vom Standpunkt der Vererbung aus einige Probleme über die soziale Klassenbildung berührt, und zwar teils um zu beleuchten, dass die verwickelten Fragen auf diesem Gebiete weit davon entfernt sind gelöst zu sein, und dass sie schwer zu untersuchen sind, teils auch um zu betonen, dass auf der Basis unserer jetzigen Kenntnisse es nicht möglich ist, Aussagen über erbliche Unterschiede zu machen, die eventuell zwischen den Klassen vorliegen. Das Problem ist sehr kompliziert und unser Wissen so mangelhaft, dass es nicht möglich ist, einen Standpunkt im Hinblick auf die Fragen über die soziale Organisation, ausgehend vom Fehlen oder Vorhandensein spezieller erblich bedingter Unterschiede zwischen verschiedenen sozialen Schichten, zu motivieren. Der Vollständigkeit halber soll schliesslich erwähnt werden, dass man selbstverständlich die Probleme nicht ausschliesslich vom Standpunkt der Vererbung aus ansehen kann. Gesichtspunkte organisatorischer, ökonomischer Art u. s. w. müssen selbstverständlich auch beachtet werden.

Ein anderer Fragenkomplex, der ebenfalls die Gattenwahl und die Isolatgrenzen berührt, bezieht sich auf die Rassenunterschiede. In den meisten Fällen kann man eine Rasse als eine Gruppe von Isolaten bezeichnen, die regional begrenzt sind und erblich bedingte Unterschiede anderen Isolaten gegenüber aufweisen. Spielen Vorstellungen über die Vortrefflichkeit der eigenen Rasse und Unterlegenheit anderer Rassen herein, so kommt indessen auch nicht selten eine Gattenwahl hinzu. So haben z. B. die Juden in gewissem Ausmasse regional begrenzte Isolate ausgemacht. Sie haben aber auch soziale Isolate dargestellt, und man kann, wenn man so will, geltend machen, dass in der Weise eine Gattenwahl vorgelegen hat, als Juden in grösserem Ausmasse Ehen miteinander als mit Nicht-Juden geschlossen haben und umgekehrt. Auf ähnliche Weise führten die Gegensätze zwischen Weissen und Neger in Amerika zu Isolatgrenzen, die man in gewissem Masse als Ausdruck für eine Gattenwahl ansehen kann.



geringerer Bedeutung, wenn ein grosser Teil der Variationsbreite, der die beiden Gruppen, die verglichen werden sollen, auszeichnet, beiden gemeinsam ist. In dieser Arbeit wird eine Methode referiert, die darauf ausgeht, ein Mass für Rassenunterschiede zu erhalten, und die im Prinzip darin besteht, dass man in erster Linie die Variationsbreite für die verschiedenen Eigenschaften in den Gruppen, die man vergleichen will, untersucht. Hernach stellt man sich vor, dass man die beiden Gruppen miteinander vermischt, worauf man versucht, die Individuen mit Hilfe der Kenntnisse, die man über sie erworben hat, zu unterscheiden. Im besten Falle können alle Individuen auf die richtige Weise heraussortiert werden. Sind die Unterschiede geringer, erhält man eine grössere oder kleinere Restgruppe, die man nicht mit Sicherheit der einen oder der anderen Rassengruppe zuteilen kann. Die Grösse dieser Restgruppe stellt ein Mass für den Rassenunterschied dar.

Man kann selbstverständlich die Methode dazu anwenden, um die Grössenordnung von Unterschieden zwischen Materialgruppen festzustellen, auch wenn es sich um andere Eigenschaften als Rassenunterschiede handelt.

Die Methode ist von besonderem Wert, wenn es sich um unsicherere Rasseneinteilungen handelt, d. h. wenn die Unterschiede klein sind. Ein Vorteil dieser Methode ist, dass man gezwungen ist, auf die Kenntnisse, die man faktisch über die betreffenden Rassengruppen hat, zurückzugreifen, und nicht unbestimmtere und deswegen zweifelhafte „Beobachtungen“ anwenden kann; gleichzeitig wie man völlig die exakten Kenntnisse benutzt, die man hat. Bisher ausgeführte Untersuchungen über Rassen sind in vielen Fällen zu mangelhaft, um eine Analyse nach der hier angegebenen Methode zu erlauben. In dem Masse, in welchem die Methode angewandt werden wird, dürfte sie indessen dazu beitragen können, dass die Rassenforschung einen objektiveren Charakter erhält, und dem veralteten Aberglauben entgegenwirken, der gegenwärtig mancherorts als wissenschaftliche Rassenforschung angesehen wird.

Im Vorgehenden wurden einige Anwendungen der mathematischen Theorie für die Populationen vom Gesichtspunkt der Vererbung aus angedeutet, die in dieser Arbeit aufgestellt wurde. Es wurde beabsichtigt, Interesse für Probleme dieser Art zu wecken und hierdurch zu versuchen, die wissenschaftliche Einstellung auf diesem Gebiete zu beeinflussen. Es dürfte unnötig sein zu unterstreichen, dass in

der hier gegebenen übersichtlichen Darstellung die Probleme nur andeutungsweise behandelt werden konnten und dass der Verfasser die Wahl so getroffen hat, speziell solche Fragen zu behandeln, mit welchen er selbst Berührung gehabt hat.

Schliesslich soll hervorgehoben werden, dass die mathematische Analyse der Bevölkerungen vom Standpunkt der Vererbung aus zeigt, dass eine Reihe von Schlüssen, die man früher mit Hilfe von Analogien aus der Tier- und Pflanzenwelt ziehen zu können glaubte, fehlerhaft waren und dass deswegen gewisse Vorschläge über soziale Massnahmen schlecht begründet waren. Im Hinblick hierauf dürfte Anlass bestehen, auch dann, wenn es sich um die Konsequenzen der mathematischen Populationsanalyse handelt, zurückhaltend zu sein. Die Ergebnisse, die man mehr oder minder bestimmt vermuten kann, sollen nicht als sichergestellt angesehen werden, bevor man empirisch eine Bestätigung für sie erhalten kann. Erst dann kann Anlass bestehen, sie als Ausgangspunkte für eine Besprechung der praktischen Massnahmen zu wählen.



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